IMPROVEMENTS IN SEEDED AND SEEDLESS WATERMELONS USING MALE STERILITY AND WATERMELON MOSAIC VIRUS-2 RESISTANCE

By

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A genetic male sterile tetraploid watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) line was developed by the backcross method using an unreleased inbred (SP90-1) as the recurrent parent. The BC_3-F_2 plants were used to produce triploid seed. In field trials, the experimental triploid was equivalent to the variety 'Flordalee' in fruit weight, fruit number, early and total yield, rind thickness, rind necrosis and the incidence and number of hard seeds per melon. There were differences in the sugar content, flesh color and texture, and hollowheart incidence. Using two diploid BC_3-F_2 populations, no linkage was found between male sterility and sugar content, flesh color, flesh

texture, or hollowheart. The development of tetraploid male sterile watermelon plants should lower the cost of triploid seed production, expand the germplasm base used for triploid variety development, allow for the production of 100% triploid seed and fruit, and protect the development of proprietary tetraploid germplasm.

In diploid and tetraploid watermelon lines that were tolerant to Watermelon Mosaic Virus-2 (WMV-2), tolerance was expressed in three aspects: (1) lower percent infection, (2) lower viral titer, and (3) significant runner to runner differences in viral concentration within the tolerant plants. Tolerance may be the result of several actions including inhibition of infection, replication, or movement. When challenged with WMV-2 in field trials, susceptible diploid, triploid, and tetraploid lines had a 30-35% yield loss whereas the corresponding tolerant lines did not show any yield loss. Diploid hybrids between the tolerant and susceptible lines were intermediate in yield loss, and all triploids except tolerant x tolerant showed significant yield loss. This indicates that both parents will need to be WMV-2 tolerant in order to have fully tolerant hybrids. The sugar content, hollowheart, hard seed number, rind thickness and flesh color of both susceptible and tolerant lines were generally unaffected by WMV-2.

CHAPTER 1
INTRODUCTION

Triploid Seed Production with Male-sterile Tetraploids

Watermelons (Citrullus lanatus (Thunb.) Matsum. & Nakai) are grown on 40,000 acres throughout Florida, the fourth largest producing state, and on 230,000 acres nationally (Allred & Lucier 1990). The U.S. ranks fifth in the world behind China, Turkey, U.S.S.R. and Egypt in tonnage (Allred & Lucier 1990). Worldwide, the majority of watermelon acreage is planted with seed of open-pollinated varieties, which currently costs about $10-20 per pound. In the U.S. the recent trend in watermelon varieties has been toward F_1 hybrids; F_1 seed costs $150-200 per pound (Maynard, personal communication). Triploid seedless hybrids are also increasing in popularity with consumers (Maxwell 1992). The cost of triploid seed is $150-200 per 1000 seeds or approximately $1000 per pound. The high cost of triploid seed has hampered acceptance of seedless varieties by growers who prefer a low input approach to a high risk business.

In an effort to reduce the cost of producing triploid plants, two approaches have been made. Rather than lower seed costs, generating transplants via tissue culture has been employed in an attempt to lower the cost per plant. To date this has not been economically successful. The other approach has been to identify sources of either marker genes to distinguish hybrid from non-hybrid plants at the seedling stage, or male sterility, to incorporate into the female parents of both diploid and triploid hybrids. Either approach would eliminate the need for hand labor in making pollinations.

Production of seed of the majority of triploid seedless watermelon varieties has been based on a scheme in which light green tetraploids serve as the seed parent, and a striped diploid is used as the pollen parent (Andrus et al. 1971, Kihara 1951). Thus, true F_1 hybrid plants show the dominant trait, fruit stripes, and self or sib-pollinated fruit remain with solid light green color. There are two basic methods of producing triploid seed. First, tetraploids and diploids are planted in adjacent rows, and the tetraploids are allowed to open-pollinate. Slower growth of tetraploid pollen results in a majority of the seeds harvested from the tetraploid fruit being triploid (Andrus et al. 1971, Kihara 1951). Alternatively, the diploid pollen can be transferred to the tetraploids by hand pollination,

which results in a greater percentage of triploid seed, but is also labor intensive and expensive. When using fruit rind patterns to distinguish F_1 plants from S_1 plants, the triploid fruit will be striped and seedless, whereas any fruit resulting from self- or sib-pollination of the tetraploid plants would be light green and seeded.

There are several problems associated with the fruit pattern scheme. First, there are only a few light green diploid varieties with acceptable characteristics from which to make tetraploids. This has led to a relatively narrow germplasm base and a limited number of triploid combinations for selection as potential varieties. Secondly, there is little investment incentive to create new tetraploid lines with light green fruit since a small percentage of the seed produced and sold as triploid is actually tetraploid. The tetraploid plants are identifiable by the solid light green fruit. Thus, years of tetraploid development may be obtained for the purchase of a few seed. The costly alternative of hand pollination is the only way to produce pure triploid seed. Additionally, the grower who produces triploid seedless fruit due to their premium price has a percentage of tetraploid melons, which are not marketable at the premium price. Lastly, in situations where dark skinned melons are desired, it is difficult to distinguish the diploid, triploid and tetraploid melons from one another.

This results in the occasional mislabeling of tetraploid fruit (with seeds) as seedless. The consumer is disappointed after paying a premium price for a seeded watermelon.

The development of commercially successful male-sterile parents should reduce the cost of production and market price of hybrid watermelon seed. This should stimulate a greater acceptance of triploid seedless watermelons. Additionally, the other technological, economic, and consumer problems mentioned above should be alleviated. This study compares a recently introduced seedless watermelon variety 'Flordalee' with an experimental one designed to perform like 'Flordalee' but produced using a male sterile tetraploid. Fruit yield and quality of the two varieties will be compared. These experiments test the feasibility of using male sterility for producing hybrid seed and determine if further backcrossing is needed for the development of the male sterile tetraploid.

Watermelon Mosaic Virus-2 Resistance

Since diploid and triploid hybrids are increasing in market share, it is necessary to determine whether both parents must be resistant to a disease in order to produce a resistant hybrid. Resistance to several major watermelon pathogens has been identified and introduced into commercial open-pollinated and diploid hybrid varieties. The list of

triploid varieties with tolerance to these pathogens is fairly short. Among these pathogens are Fusarium oxysporum (Fusarium wilt) and Glomerella cingulata var. orbiculare (anthracnose). However, no commercial varieties have been introduced with resistance to any of the viruses that infect watermelon (Demski & Sowell 1970, Sowell & Demski 1969). One of the major factors limiting watermelon production is the yearly occurrence of viral epidemics (Shimotsuma 1965). Three members of the potyvirus family are the most prevalent viruses in watermelon fields worldwide (Anderson 1951,1952; Purcifull et al. 1988). The first is Watermelon Mosaic Virus-1 (WMV-1), now known as Papaya Ringspot Virus type W (PRSV-W). The other two are Watermelon Mosaic Virus-2 (WMV-2) and Zucchini Yellow Mosaic Virus (ZYMV) (Antignus et al. 1989). These viruses cause systemic symptoms such as leaf mosaic, leaf distortion, fruit distortion, and reduction of both fruit quality and yield (Castle 1992, Purcifull & Hiebert 1979). These viruses are spread primarily by multiple species of aphid vectors (Bhargava & Bhargava 1976, Mora-Aguilera & Webb 1993, Webb 1992). Chemical and cultural controls have not adequately controlled either the vector or the virus (Adlerz & Crall 1967, Adlerz & Evert 1968, Webb & Linda 1993). Therefore, identification of resistant germplasm and development of cultivars with resistance to

members of the potyvirus family have been major objectives of cucurbit breeding programs (Webb 1977).

Although mosaic diseases have been observed in watermelons since the 1930s (Walker 1933), they were not named until 1960. However, the distinction between WMV-1 and WMV-2 based on host range and serology was not clearly established until 1965 (Anderson 1954). The first report identifying sources of resistance was in 1967 (Adlerz & Crall 1967) with subsequent reports in 1977, 1984, 1986, and 1993 (Gillaspie & Wright 1993, Munger et al. 1984, Provvidenti 1986, Webb 1977). Resistance to WMV-2 is reported to be controlled by a single dominant gene (Webb 1977), but no formal studies have been published to demonstrate this. Additionally, not all WMV-2 strains react the same (Bhargava & Bhargava 1976, De Sa & Kitajima 1991). To date, no WMV-2 resistant watermelon varieties have been released although several university and private breeding programs have intensive efforts to develop resistant varieties.

One goal of this study was to determine the effect of polyploidy on resistance to the viral pathogen WMV-2. There are two ways that polyploidy could affect the interaction of virus with the plant. First, expression of WMV-2 resistance could be different at the three possible ploidy levels for commercial varieties (diploid, triploid and tetraploid).

Second, morphological differences such as leaf thickness and cell size could affect the feeding behavior of aphids or the ability of aphids to transmit the virus (Lopez-Abella et al. 1988, Porter & Melhus 1932).

A second goal of this study was to determine how many resistance alleles are needed to confer commercially adequate resistance to WMV-2 in diploid and triploid cultivars. For example, will only one dose of resistance from the male parent confer resistance to the triploid? Alternatively, will only two doses from the female be enough? Possibly, resistance will be needed in both parents in order for the triploid to be fully resistant. The requirements will determine whether or not tetraploid breeding lines with WMV-2 resistance are needed.

If WMV-2 resistance proves to be a single gene dominance (as is common with viral resistance), this would be the most advantageous result. It is possible that the putative gene for WMV-2 resistance, if incorporated into diploid inbreds that were used as the male parent, could confer adequate field resistance to the triploid hybrid regardless of the susceptibility of the tetraploid seed parent. One consideration in releasing varieties with single gene resistance is the longevity of the resistance.

It has been repeatedly shown that pathogens often develop new strains that overcome single gene resistance.

This study had the following objectives:

1. Quantify the losses caused by WMV-2 to susceptible diploid, triploid and tetraploid watermelon lines.
2. Examine how effective the WMV-2 resistance is in terms of reducing viral infection and maintaining fruit yield and quality.
3. Determine whether there are any effects of polyploidy on disease resistance or susceptibility in parental lines of intended hybrids.
4. Test diploid and triploid hybrids for resistance to WMV-2 and determine whether there is any gene dosage effect.

The information derived from the investigations will give an estimate of losses by susceptible varieties under local field conditions and determine the effectiveness of resistance to WMV-2 in these lines. Additionally, the effects of polyploidy and gene dosage on resistance/susceptibility to WMV-2 will be determined. These data will inform plant breeders concerning which parents must be resistant in order to produce WMV-2 resistant varieties in hybrid or open-pollinated systems.

CHAPTER 2
TRIPLOID SEED PRODUCTION WITH MALE-STERILE TETRAPLOIDS

Literature Review

Using morphological marker traits to identify hybrid plants

Several morphological traits of watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) have been tested to determine their suitability in a phenotypic marker system to distinguish hybrid from parental strains. The non-lobed leaf trait, which is controlled by a single recessive gene (Mohr 1942), was first reported in the variety 'Georgia Rattlesnake' (Schaffner 1903). Lobing is incompletely dominant (Mohr 1942). This non-lobed trait was used to identify the percentage of outcrossing between diploids in three different plant spacings and patterns (Mohr et al. 1955). Three-week-old seedlings were used to distinguish F_1 lobed hybrids from non-lobed non-hybrids. This trait was successfully used to identify the F_1 hybrid plants at the three-week-old stage. The degree of outcrossing ranged from 20% to 36%. This degree of hybridization is insufficient to use natural outcrossing for the production of diploid

hybrids, as excessive seeding and thinning would be needed. This trait has not been used in outcrossing studies between tetraploids and diploids. However, reports of the amount of outcrossing between tetraploids and diploids range from 67% to 83%, depending on the arrangement of the plants (Andrus et al. 1971).

The dwarf or short internode trait first reported in 1956 (Mohr 1956) is another morphological trait used to identify hybrids. This trait is controlled by a single recessive gene (Mohr 1956). Two apparently pleiotropic effects of this gene were reported: slightly curved seeds and delayed petal development in staminate flowers (Mohr 1958, 1963). Dwarf habit has been successfully used to distinguish hybrids from non-hybrids. However, the previously mentioned problem of seeds produced by outcrossing on tetraploid seed parents would remain significant. Although exploitation of morphological markers would solve the farmers' and consumers' problems of having unidentified tetraploids in the field and seeded melons, the seed company would still face losing their proprietary tetraploid germplasm.

A third morphological marker for identifying hybrids is the yellow leaf character first reported by Porter in 1937 (Filor 1939, Porter 1937a). Green leaf color was reported

to be dominant over yellow. Barham (1956) reported that this character for chlorotic leaves was controlled by a single recessive gene. However, Warid and Abd-El-Hafez (1976) reported that F_1 plants from a cross between the varieties 'Congo' (green leaf) and 'Yellow Skin' (yellow leaf) had pale yellow foliage, demonstrating incomplete dominance. Additionally, using a green leaf tetraploid and a yellow leaf diploid produced triploid plants which were pale yellow, further supporting the incomplete dominance of the green leaf trait (Warid et al. 1971). This disagreement over inheritance may be due to different genes for yellow leaf color because no test for allelism has been reported. The variety 'Yellow Skin' in Warid's study (1971) was derived from an unnamed Japanese variety, whereas Barham (1956) used the cultivar 'Royal Golden' and Filor (1939) used the cultivar 'Selo-Koryi'. The evaluation for leaf color was made at first female flower, which would be too late for practical use. Ideally, whatever trait is used to identify hybrids should be expressed in the early seedling state of development.

Two different seedling markers have been reported by Rhodes (1986) and Zhang et al. (1996). One is juvenile albino (*ja*), in which cotyledons of seedlings are yellowish to creamy and leaves gradually turn greenish-yellow with

white edges. This trait was found to be a monogenic recessive and unlinked to any of the known male-sterile genes (Rhodes et al. 1988). The other gene is termed delayed-green (dg). Plants expressing this trait have yellow cotyledons and pale green newly developed leaves (Zhang et al. 1996). This trait was also found to be monogenic recessive (Rhodes et al. 1988). Fortunately, both of these traits allow identification of hybrids at the seedling stage. Therefore, they are of use in facilitating cross-pollination by allowing plants with the ja or dg phenotype to open-pollinate and subsequently identifying hybrids as non-ja or -dg before transplanting to the field. However, as with nl, dw, and yellow leaf, both ja and dg are unlinked to male-sterility; therefore, they do not provide the advantage of distinguishing male-fertile from male-sterile plants prior to transplanting or flowering.

Using chemically induced male sterility for hybridization

An alternative to using morphological markers to identify hybrid plants is the development of male sterility, which is commonly used in a number of vegetable variety development programs (Driscoll 1986, Kaul 1988, Rao et al. 1990). The use of male sterility solves the problem of producing parental selfs and protects the proprietary rights

to tetraploid germplasm because, theoretically, 100% of the seeds produced should be hybrid.

Prior to any reports of genetic male sterility, chemical growth regulators were screened for their ability to induce male sterility. This principle of chemical induction of male sterility has been widely reported and used (Rhem 1952, Wittwer 1953). One compound, sodium alpha, beta-dichloroisobutyrate, was reported to eliminate the opening of staminate flowers, while the production of abundant, viable pollen was unaffected. The ratio of functional male:female flowers was 10:31 in treated plants, whereas in untreated plants, it was 675:45. No changes in female fertility with respect to seed and fruit set were found. No report of the percentage of outcrossing was given (Hensz and Mohr 1959). To date, no further exploration or utilization of this approach has been reported.

Genic male sterility with the glabrous marker

The use of genetic male-sterile lines has been reported in a variety of crops (Driscoll 1986, Kaul 1988, Rao et al. 1990). The first report of a genetic male-sterile watermelon was by Watts (1962) who irradiated seed of the variety 'Sugar Baby' and found a segregant in the second generation that produced staminate buds with anthers that do

not dehisce. This male-sterile trait is controlled by a single recessive nuclear gene that apparently has a pleiotropic effect of glabrous (hairless) plants. The glabrous male sterile (originally ms, and now gms) is an ideal type of male sterility because the sterile plants are obviously different from their fertile counterparts in the seedling stage. However, the male-sterile plants were also highly sterile with respect to seed production. The glabrous male-sterile plants also had reduced vigor and soluble solids and had the undesirable horticultural traits whiteheart and hollowheart (Watts 1967). Subsequently, efforts to improve the seed setting ability, vigor, disease resistance and fruit quality were made (Watts 1967). Although an apparent linkage of Fusarium and anthracnose susceptibility to the gms homozygotes was observed, several progeny with resistance to anthracnose and Fusarium wilt were identified. The seed setting ability of the glabrous plants was improved from 0.3 seeds per fruit to 244 after 3 outcrosses. This is a significant achievement in improving fertility but is well below the outcross parent (Watts 1967). The fruit quality of these lines remained unacceptable and efforts to improve them have resulted in renewed reduction of seed productivity (Watts 1967).

The gms gene was transferred from a diploid breeding line to a tetraploid line through a series of controlled pollinations in which a tetraploid was used as the male parent and the gms diploid line as a female (Love et al. 1986). Twenty triploid plants obtained from this method were pollinated by a different tetraploid source, yielding 6 seeds, which when germinated, resulted in 4 tetraploids and 2 triploids. Selfing of these tetraploids for two generations produced several glabrous male-sterile tetraploids. These tetraploids also retained the reduced fruit quality and seed production characteristic of the diploid gms line (Love et al. 1986). To date, this gene has not been successfully used to produce either diploid or triploid hybrids due to these undesirable traits, which after 30 years at the diploid level and 10 at the tetraploid level have not been eliminated. The cause of male sterility in these lines is desynapsis of homologous chromosomes (Ray & Sherman 1988).

A glabrous male-sterile plant was identified by Dale Lee Yadon, the author and G. Elmstrom in 1992 (unpublished data) in a breeding line at Leesburg, Florida. This line produces a normal number of seeds per fruit but is not vigorous, and its disease resistance has not been evaluated. No data regarding the inheritance or gene designation have been generated or published. This line has been used to

develop diploid breeding lines that are improved in horticultural characteristics but has not yet reached the variety testing stage of development (Elmstrom, personal communication).

Genic male sterility without a seedling marker

An unmarked genetic male sterile line was identified in 1982 in China in the variety 'Xian No.2' (Xia et al. 1988). The male flowers of plants with this gene produce anthers that are extremely small and do not dehisce any pollen (Fig. 2-1).

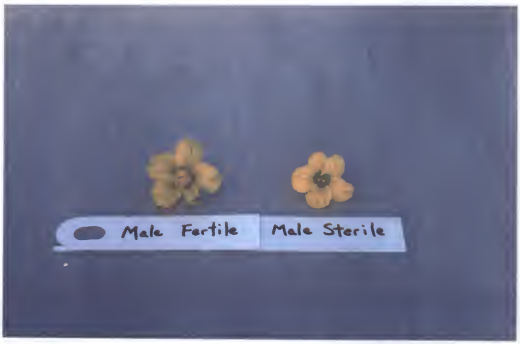

Fig 2-1. Male flowers from male-fertile and male-sterile plants. Note the vestigial anthers and absence of pollen in the male-sterile flower.

This male-sterile trait is controlled by a single recessive nuclear gene (Xian 1991)(ms), which is non-allelic to the glabrous male-sterile gene reported by Watts (Murdock et al. 1990). There are no apparent pleiotropic effects of this gene. Although the original breeding line is not Fusarium resistant, its horticultural characteristics are superior to the gms line. The flesh is red and moderately sweet (brix ~ 10.0). However, the flesh texture is softer than most American varieties, and it is slightly more prone to hollowheart. The seed production of this line is equivalent to its male-fertile counterpart. The fruit are medium in size (4 kg.), round and have dark green stripes and small brown seeds.

Maintaining genic male sterility

The technique for maintaining male-sterile parents at the tetraploid and diploid levels is given below. A tetraploid male-sterile line would be maintained by sib-pollinating a simplex male fertile (Ms ms ms ms) onto a nulliplex male sterile (ms ms ms ms). The progeny from this cross would segregate 1:1 male fertile:male sterile. All of the male fertile plants would be simplex male fertile and could be used to pollinate any male-sterile plant, with the progeny again segregating 1:1 fertile:sterile. A diploid

male-sterile line would be maintained in a similar manner. A male-sterile plant (ms/ms) would be pollinated by a heterozygous male-fertile plant (Ms/ms). The progeny would segregate 1:1 male fertile:male sterile with all of the male fertile plants being heterozygous Ms/ms and capable of pollinating any male-sterile plant with progeny segregating 1:1 fertile:sterile (Love et al. 1986).

There are no distinguishing markers to identify sterile segregants at the seedling stage, necessitating identification and roguing in seed production fields. Thus, the diploid and tetraploid breeding lines would be maintained in a manner similar to the glabrous male-sterile lines (Murdock et al. 1990, Xian 1991). A similar male-sterile source was identified in the variety 'Kamyzyakskii' (Dyutin 1990).

Genetic linkage between male sterility and fruit qualities

As with the glabrous male-sterile trait, there may be some linkage of undesirable horticultural traits with the ms gene for male sterility. The fruit quality characteristics for which 'Rubylee' was not equivalent to 'Flordalee' were sugar content, flesh color and texture, and possibly hollowheart. No articles concerning the inheritance of flesh texture or hollowheart were found, but several reports were found concerning linkage of sugar content to either

flesh color (Abd-El-Hafez 1985) or yield (Sidhu 1982) of watermelon.

Genetics of fruit quality

There are several reports on the inheritance of total soluble solids, which give the approximate sugar content. Chambliss (1966) stated that a large number of genes controlled this trait and that there was dominance toward low sugar content. A second report found only one gene pair controlling total soluble solids (Chung 1967). A third report found three incompletely dominant genes and at least two minor genes (Suzuki & Hall 1971). A fourth report found between one and three gene pairs governing this trait (Abd-El-Hafez 1985). Several other reports give conflicting conclusions regarding the direction of dominance (Barna 1965, Brar & Nandpuri 1977, Filov & Toscev 1968, Abd-El-Hafez 1983, Ivanoff & Albritton 1963). However, most authors agree that there are highly significant additive effects (Brar & Nandpuri 1974, Brar & Nandpuri 1977, Dhaliwal 1984, Sidhu 1977).

Flesh color is one of the major attributes by which consumers evaluate watermelons. Deep red is generally preferred over lighter shades. There are several reports on the genetics of watermelon flesh color. White flesh has been reported as epistatic to a second gene which conditions

yellow and red flesh (Shimotsuma 1963). Red is dominant to yellow flesh of the 'Golden Honey' type, but Canary yellow is dominant to pink. Red is epistatic to orange flesh, which in turn is epistatic to yellow from 'Golden Honey' (Abd-El-Hafez 1981, Henderson 1989, Poole 1944, Porter 1937b). However, data from a cross between white and red-fleshed lines have segregated in more complex patterns. Segregants included the parental types, white and red, but also included yellow. Additionally, the distribution of color varied within a fruit, e.g., localization of red or yellow to the tissue surrounding the seeds, whereas the remainder of the flesh was white, vs. uniform color distribution throughout the fruit, or red and yellow localized in different zones. This suggests a complex inheritance of color pattern distribution in melon fruits (Navot et al. 1990).

The red flesh color of watermelons is mainly due to lycopene accumulation. This is similar to other crop plants such as tomato. A study of the carotenoid content of red-fleshed watermelons found that the degree of redness ranging from pink to deep red was due to quantitative differences in lycopene content (Tomes et al. 1963). Another study examining the carotenoid content of red, orange, and yellow-fleshed watermelons found that orange flesh was due to high B-carotene concentration, whereas this yellow was due to

lower levels of B-carotene plus xanthophyll. Lycopene was the major constituent of red flesh although other carotenoids were also found (Watanabee & Saito 1987).

It may be possible to formulate some hypotheses about watermelon flesh color based on the well established biosynthetic pathways and both traditional and molecular genetic analysis of its control in tomato and other species (Pecker et al. 1996, Tomes et al. 1963, Watanabe & Saito 1987).

Genetic linkage in watermelon

The pleiotropic effects of the glabrous male sterile gene have been previously mentioned. Additionally, the absence of linkage between the chlorophyll mutants juvenile albino, delayed-green, spotted and yellow and male sterility was also stated (Rhodes et al. 1988, Zhang et al. 1996). There is a report of linkage between flesh color and sugar content (Abd-El-Hafez 1985). The study used crosses between varieties of orange vs. white-yellow fruit flesh color. In both cases, low sugar content was completely associated with all white-fleshed segregants (Abd-El-Hafez 1985). Another report found an association between high yield and lower sugar content, including the converse low

yield and high sugar content. However, it was added that the association was not so strong that selection for both would be incompatible (Sidhu 1982).

The first linkage map for watermelons was based primarily on isozymes. This map consisted of 7 linkage groups with 24 loci (Navot et al. 1990) Since the chromosome number of watermelon is 2n=22, substantial portions of the genome are not covered by this map. More recently, a map based primarily on random amplified polymorphic DNA (RAPD), but including RFLP, isozyme and morphological markers, was constructed (Hashizume et al. 1996). A total of 62 loci were mapped to 11 linkage groups covering 524 centimorgans. Included on this map was a linkage between a flesh color gene (w) and a RAPD marker on linkage group 6.

Materials and Methods

Development of genetic male sterility in watermelon

To develop a tetraploid male-sterile breeding line, seven-day-old seedlings of the diploid line G17AB developed and described by Xia et al. (1988) that were segregating 1:1 fertile:sterile were treated with aqueous colchicine following the method of Kihara (1951). Putative tetraploids

were identified by counting chloroplasts in 30 stomatal guard cells (Ho et al. 1990). These tetraploids were transplanted into the breeding field at the research farm in Leesburg, FL, at the direction of Dr. G. Elmstrom and all breeding and subsequent selections were carried out by him.

Male-sterile tetraploid plants were either sib-pollinated with male-fertile tetraploids or crossed with existing tetraploid breeding lines developed by Dr. Elmstrom including SP90-1. The progeny of the male sterile sib-pollinations were grown, and sterile segregants were either sib-pollinated with fertile segregants or crossed with a diploid breeding line to generate triploid seed.

Development of a tetraploid male-sterile maintainer line

The progeny of the second sib-pollination were examined for segregation ratios. Sterile segregants from lines which were segregating 1:1 fertile to sterile were sib-pollinated with the simplex male-fertile plants. The progeny of this cross will again segregate in a 1:1 fertile to sterile ratio and are used as a maintainer line.

Triploid performance by the male-sterile tetraploid

To test whether the male-sterile tetraploid could be used directly as a parent, Dr. Elmstrom crossed it with the variety 'Small Seeded Dixielee' (SSDL)(Crall et al. 1994),

which has been used to produce several other good quality hybrids. Plants of both the diploid and tetraploid male-sterile breeding lines were grown in a field trial that included the triploid cross between the male-sterile tetraploid and 'SSDL'. Dr. Elmstrom, his technician, and the author harvested fruit from the diploid, tetraploid, and triploid plants and evaluated them for weight, size, sugar content, flesh color, hollowheart, and seed number.

Incorporation of male sterility into existing tetraploids

To introduce the male-sterile trait (ms) (Zhang & Wang 1990) into existing high quality tetraploids, the F_1 progeny of crosses between the male-sterile tetraploid and tetraploid breeding lines, one of which was SP90-1, were grown and self-pollinated by Dr. Elmstrom. The F_2 progeny were grown and male-sterile segregants were backcrossed to the recurrent parent SP90-1 by Dr. Elmstrom. The procedure of selfing the BCF_1 and backcrossing the sterile F_2 segregants to SP90-1 was repeated two more times by Dr. Elmstrom.

To determine whether the BC_3-F_2 male-sterile version of SP90-1 was sufficiently equivalent to the recurrent parent, the author made the following two crosses: SP90-1 X SSDL and ms BC_3-F_2 SP90-1 X SSDL. The former cross is the recently introduced triploid variety 'Flordalee'. The latter cross

was named 'Rubylee' for the purposes of this experiment.

Field trial procedures

Three locations (Ruskin, Leesburg, and Gainesville) were used to compare 'Flordalee' and 'Rubylee'. Transplants for the Ruskin location were grown by the Speedling Company. Transplants for the Leesburg and Gainesville location were produced by planting seeds of each in 72-cell-size Speedling flats using sterile potting mix. The flats were kept under greenhouse conditions and fertilized once per week with 20-20-20. Seedlings at the 3-5 true-leaf stage of development were transplanted to the field. The rows were raised beds 15 cm high and 60 cm wide covered with black polyethylene mulch. Methyl bromide:chloropicrin (98:2) (448 kg/ha) was applied to the beds when they were covered with plastic mulch. In Gainesville 740 kg/ha of 10-10-10 was applied pre-plant and an additional 41 kg/ha of N (NH_4NO_3) was applied through the drip irrigation system. Fungicides (Bravo 720 3507 ml/ha, Kocide 2.24 kg/ha, Maneb 2.67 l/ha, and Topsin .56 kg/ha) and herbicides (Alanap 4.48 kg/ha, Dual 876 ml/ha, Paraquat 2.34 l/ha, Prefar 5.6 kg/ha, and Roundup 1.12 kg ai/ha) were applied 1-2 times per week in accordance with the recommendations of the Florida Watermelon Production Guide (Hochmuth & Elmstrom 1992).

The Gainesville field plots were five rows wide with

the test plants on the outside rows and the variety 'Mickylee' used as a pollinizer on the interior rows. Each variety was replicated 25 times with 10 plants per replicate. Row spacing was 2.44 m apart and plant spacing was .91 m within the row with 1.82 m spacing between plots in the row.

The Leesburg field plots were four rows wide with the variety 'Mickylee' used as pollinizer. The test plants were on the outside rows with the pollinizers on the inside rows. Each variety was replicated 25 times with 10 plants per replicate. Row spacing was 2.74 m apart and plant spacing was .91 m apart with 1.82 m spacing between plots in the row. The fertilizer applied pre-planting to the Leesburg field was 900 kg/ha of 6-8-8. Liquid fertilizer 6-0-8 was injected three times per week for twelve weeks at a rate of 4.6 l/ha. The fungicide Bravo 720 was applied once per week at 3507 ml/ha.

In Ruskin, the plots were three rows wide with the test plants on the outside rows, and the variety 'Mickylee' was planted on the inside row. Each variety was replicated 13 times with 10 plants per replicate. Row spacing was 1.82 m apart and plant spacing was 1.22 m within the row with 2.44 m spacing between plots in the row.

Variety evaluation procedures

Fruits from each location were harvested 30-35 days after fruit set, and the following data were taken: fruit number, individual fruit weight and size, sugar content, rind thickness, flesh color, taste and texture, hollowheart rating and seed number. The flesh color, taste and texture were scored on a subjective basis by the author and the harvesting crews. The data for the two varieties were compiled and compared by location. The statistical method used for detecting differences in fruit number, fruit weight, fruit size, soluble solids (sugar content), and rind thickness, was Duncans new multiple range. The statistical method used for incidence and size of hollowheart, the percent of fruit with seed, and the number of seeds per fruit was Fishers Exact Test. This was used due to the low frequency of occurrence of fruit with these characteristics. The comparison of hollowheart in fruit from male-fertile plants versus fruit from male-sterile plants was by a binomial test of proportions. The distribution of numbers of fruit classified by flesh color, flesh texture, and flavor was analyzed by the Mann-Whitney test for ranked data. All tests used the SAS software.

Linkage of male sterility to horticultural characteristics

Based on the results of these three trials, a follow-up study was performed to examine if there was any linkage between male sterility and any of the following traits: flesh color, sugar content, flesh taste and texture, and hollowheart. Because specific linkages were sought, more advanced breeding lines were used rather than the normal F_2 lines. Two population were used to examine these questions. One population was a BC_3-F_2 diploid line segregating for male sterility with 'SSDL' as the recurrent parent. The second population was a BC_3-F_2 diploid line segregating for male sterility with 'Jubilee II' as the recurrent parent. Both of these lines were developed at the University of Florida by Dr. Elmstrom using the material developed by Xia as the source of male sterility.

Transplants of each line (400-600) were established in the field at the Horticulture Research Unit in Gainesville. At flowering, plants were marked as being either male fertile or male sterile. Fruits from fertile and sterile plants were harvested separately at approximately 35 days after setting. Each fruit was evaluated according to the criteria previously stated. Each characteristic of the fruit from male-sterile plants was then compared to those from male-fertile plants and analyzed as previously described.

Results and Discussion

Fruit yield

One of the most critical aspects of a watermelon variety is total yield. The yield of 'Rubylee' was equivalent to that of 'Flordalee' at all three locations (Table 2-1). This includes early, late and total yields. There were two instances where yield components differed. At Gainesville, 'Rubylee' had a higher number of fruit per plot in the early harvest; however, the early yield was not different (Table 2-1). At Leesburg, 'Flordalee' had a higher average fruit weight in the 2nd harvest but did not differ from 'Rubylee' in fruit yield in the 2nd harvest (Table 2-1). Additionally, no significant differences were found between 'Rubylee' and 'Flordalee' in other important traits, viz., incidence of rind necrosis, rind thickness, incidence and number of hard seeds per melon (Table 2-2).

Fruit quality

There were significant differences between 'Rubylee' and 'Flordalee' at all three locations in the following traits: soluble solids (sugar content), flesh color, texture and taste. 'Rubylee' was lower in sugar content than 'Flordalee' (Table 2-2). The flesh color of 'Rubylee'

fruit was the same as 'Flordalee' in 10-20% of the fruit, whereas the remainder of the 'Rubylee' fruit were a lighter red in color. The flesh texture of 'Rubylee' was generally more fibrous or chewy than 'Flordalee'. The flavor of some 'Rubylee' fruit was not as good as 'Flordalee'. The incidence and severity of hollowheart was greater in 'Rubylee' when compared to 'Flordalee' at 2 out of 3 locations. Because differences in several key characteristics were found, some changes were initiated in the original plan. The germination of 'Flordalee' versus 'Rubylee' and seed set in hand versus open-pollinated fruit was dropped, and a linkage study was added.

Linkage of male sterility to fruit quality

In the BC_3-F_2 population with 'Jubilee II' as the recurrent parent, fruit from 600 plants were separated on the basis of source: harvested from male-sterile or male-fertile plants. There were no significant differences between fruit from sterile and fertile plants in the following traits: incidence and severity of hollowheart, sugar content, flavor, flesh color, and texture (Tables 2-3, 2-4, 2-5, 2-6, 2-7).

In the BC_3-F_2 population with 'SSDL' as the recurrent parent, fruit from 500 plants were separated on the basis of source: harvested from male-sterile or male-fertile plants.

There was no significant difference between fruit from the male-sterile versus male-fertile plants in the overall sugar content, incidence and severity of hollowheart, flesh color and texture, and flavor (Tables 2-8, 2-9, 2-10, 2-11, 2-12).

Therefore, it can be concluded from these experiments that there is no linkage association between male sterility and the following traits: sugar content, incidence and severity of hollowheart, flesh color and texture, and flavor.

Evaluation of male sterility to produce hybrid watermelons

The variety trials between 'Rubylee' and 'Flordalee' show that male sterility is effective in producing 100% triploid seed because no tetraploids were observed in the 'Rubylee' plots. Additionally, by eliminating the need for hand pollination, male sterility should be effective in lowering the cost of seed production.

Although 'Rubylee' was not equivalent to 'Flordalee' in all characteristics, the experiments using the diploid lines to look for linkage between male sterility and several undesirable fruit quality traits show that there are no negative linkages that must be broken. It should be noted that the selection pressure on the tetraploid F_2 population segregating for fertility was not very high due to the low numbers of sterile plants expected (on average 1/36). Traditionally, the backcross method of breeding would use at

least 5 backcrosses to incorporate a particular trait into an existing variety. These experiments show that the system of male sterility has great potential for the production of hybrid watermelon seed, but that further backcrossing with selection will have to be done on this particular tetraploid line. Effective selection will only be possible if large F_2 populations are employed to generate significant numbers of male-sterile tetraploids.

Currently, a phenotypic marker system is used to produce triploid seed and fruit. In this system, unpatterned light green tetraploids are crossed with striped diploids to produce triploid seed. In the subsequent production of fruit the triploid seedless fruit is striped, whereas any non-striped fruit is tetraploid and seeded.

If the tetraploid population segregating 1:1 male fertile: male sterile is properly rogued, there is no need for a phenotypic marker system as the male-sterile tetraploid will produce 100% triploid seed. In the three field trials, there were no tetraploids found among the 650 'Rubylee' plants.

If a 100% pure triploid stand can be consistently achieved, the farmer will benefit because there will be no tetraploid seed, for which he paid the premium triploid price; and no field space will be wasted on the production of tetraploid fruit, which will not bring the same price as

the triploid. The consumer will also benefit because no seeded fruit will be mistakenly marketed as seedless. An additional benefit to the farmer should be a lower cost of triploid seed, provided that bee pollination produces triploid seed of equal quality and quantity as hand pollination. Another benefit of producing 100% triploid seed is that the tetraploid parent may be kept proprietary rather than giving it away with the triploid seed. This should stimulate additional investment in triploid variety development. The last major benefit of using male-sterile tetraploids for seed production is that since the phenotypic marker system is not necessary, additional fruit types with a wider genetic base may be used to develop new triploid combinations. One feature this male sterile gene lacks is a linked seedling marker gene to facilitate roguing male fertile segregants.

A summary of the results and benefits of using male-sterile tetraploids to produce triploid seed is as follows:

- Male sterility has been introduced into a tetraploid watermelon line via the backcross method.
- This tetraploid was used to produce a triploid that was equivalent to the variety 'Flordalee' with respect to fruit size, fruit number, total yield, incidence and number of hard seeds per melon, rind thickness, and rind necrosis.
- Further backcrosses should improve the performance of the tetraploid with respect to sugar content, flesh color, flesh texture, hollowheart, and flavor.

- Male-sterile plants can be used to produce 100% hybrid seed without the need for hand pollination.
- The cost of seed production should be lower.
- Germplasm may be kept proprietary.
- These factors should stimulate additional investment by seed companies and increase profit to farmers.
- No field space will be wasted on tetraploids when producing triploid fruit.
- The germplasm base that can be used to produce triploid hybrids will be expanded by eliminating the need for a phenotypic marker system.
- Consumers will not be disappointed by purchasing a seeded melon after having paid for a seedless melon.

Table 2-1. Performance of Flordalee[z] and male sterile Rubylee[y] seedless watermelons, Fall 1996.

		Ave. no. of fruit per plot			Ave. wt. per fruit (kgs)			Ave. yield per plot (lbs)		
		Harvest no.			Harvest no.			Harvest no.		
	Hybrid	1	2	Total	1	2	Total	1	2	Total
Gainesville	Flordalee	17.5 b[x]	8.5 a	26 a	4.1 a	2.4 a	3.6 a	72.1 a	19.5 a	91.6 a
	Rubylee	20 a	8.5 a	28.5 a	3.9 a	2.4 a	3.4 a	77.1 a	19.1 a	96.6 a
Leesburg	Flordalee	7.3 a	24.5 a	31.8 a	6.8 a	4.2 a	4.8 a	49.9 a	102.5 a	152.4 a
	Rubylee	8.6 a	24.3 a	32.9 a	6.3 a	4.0 b	4.7 a	54.0 a	98.4 a	152.4 a
Ruskin	Flordalee	8.1 a	7.1 a	15.2 a	4.2 a	3.0 a	3.6 a	33.6 a	21.3 a	54.9 a
	Rubylee	8.0 a	7.1 a	15.0 a	4.1 a	3.0 a	3.6 a	32.6 a	21.3 a	54.0 a

[z]A hybrid from the cross SP90-1 x SSDL (Small Seeded Dixielee).

[y]A hybrid: the ms gene in BC_3-F_2 to SP90-1 x SSDL.

[x]Letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 2-2. Evaluation of various characters in Flordalee[z] and Rubylee[y] seedless watermelons, Fall 1996.

			Hollow heart		Hard seeds		Rind thickness and necrosis	
	Hybrid	Soluble solids %	% of fruit with	Ave. size (mm)	% of fruit with	Ave. no. /fruit	Ave. size (mm)	% of fruit with
Gainesville	Flordalee	11.74 a[x]	29 b	10.7 b	48 a	1.4 a	12.5 a	20 a
	Rubylee	11.32 b	58 a	14.0 a	40 a	1.1 a	13.0 a	27 a
Leesburg	Flordalee	11.91 a	32 b	13.8 b	36 a	1.1 a	14.3 a	10.0 a
	Rubylee	11.49 b	51 a	16.9 a	42 a	1.3 a	14.3 a	14.8 a
Ruskin	Flordalee	11.74 a	39 a	15.3 a	36 a	1.0 a		
	Rubylee	11.05 b	54 a	13.9 a	24 a	0.8 a		

[z]A hybrid from the cross SP90-1 x SSDL (Small Seeded Dixielee).

[y]A hybrid: the ms gene in BC_3-F_2 to SP90-1 x SSDL.

[x]Letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 2-3. A comparison of the percent total soluble solids from a ms BC_3-F_2 to Jubilee II population[z] segregating for male sterility.

Male fertile	Male sterile
9.0 % a[y]	9.6 % a

[z]Sample size from the fertile and sterile plants was 300 and 129 fruits, respectively.
[y]Letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 2-4. A comparison of the incidence and severity of hollowheart in a ms BC_3-F_2 to Jubilee II population segregating for male sterility.

	Male fertile	Male sterile
No. of melons with hollowheart	22	7
No. of melons examined	300	129
% of melons with hollowheart	7.3% a[z]	5.4% a
Ave. size of hollowheart (mm)	187 mm total/22 = 8.5 mm a	63 mm total/7 = 9.0 mm a

[z]Letters in common indicate no significant difference at the .05 level as determined by a binomial test of proportions.

Table 2-5. Distribution of number of fruits classified for flesh color in fruits from a ms BC_3-F_2 to Jubilee II population segregating for male sterility[z].

	Male fertile	Male sterile
Dark red	6	2
Bright red	7	7
Deep pink	16	9
Med red w. salmon[y]	1	0
Med red w. orange	6	3
Med red	35	17
Light red w. salmon	16	0
Light red w. orange	18	2
Light red	74	40
Pink w. salmon	16	0
Pink w. orange	4	1
Pink	70	14
Salmon	16	4

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.
[y]W. salmon (or orange) means that a tinge of salmon (or orange) color exists throughout the flesh, but the first color named is predominant.

Table 2-6. Distribution of number of fruits classified for flesh texture in fruits from a ms BC_3-F_2 to Jubilee II population segregating for male sterility[z].

	Male fertile	Male sterile
Crunchy	8	1
Firm	6	1
Crisp	31	14
Smooth	6	1
Gritty	4	3
Fibrous	8	10
Meaty	6	2
Rubbery	26	5
Crisp and chewy	2	1
Chewy	35	13
Soft and chewy	63	10
Soft	73	42

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.

Table 2-7. Distribution of number of fruits classified for flavor in fruits from a ms BC_3-F_2 to Jubilee II population segregating for male sterility[z].

	Male fertile	Male sterile
Excellent	6	3
Good	84	34
Cucumber/bitter	38	13
OK	96	36
Sour	22	8
Off	22	2
Weak	35	17
Bland	7	8
Poor	4	1

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.

Table 2-8. A comparison of the percent total soluble solids from a ms BC_3-F_2 to SSDL population[z] segregating for male sterility.

	Male fertile	Male sterile
1st harvest	12.1 % a[y]	12.1 % a
2nd harvest	11.2 % a	11.1 % a
3rd harvest	10.6 % a	9.2 % b
Total	11.2 % a	11.5 % a

[z]Total sample size from the fertile and sterile plants was 243 and 74 fruits, respectively.
[y]Letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 2-9. A comparison of the incidence and severity of hollowheart in a ms BC_3-F_2 to SSDL population segregating for male sterility.

	Male fertile	Male sterile
No. of melons with hollowheart	12	9
No. of melons examined	243	74
% of melons with hollowheart	4.9% a[z]	12.1% a
Ave. hollowheart size (mm)	113 mm total/12 = 9.4 mm a	115 mm total/9 = 12.7 mm a

[z]Letters in common indicate no significant difference at the .05 level as determined by a binomial test of proportions.

Table 2-10. Distribution of numbers of fruits classified for flesh color in fruits from a ms BC_3-F_2 to SSDL population segregating for male sterility[z].

	Male fertile	Male sterile
Deep red	32	3
Deep red w. orange[y]	7	1
Red	20	12
Orange red	7	7
Med red w. orange	20	4
Med red	70	14
Orange	2	1
Red w. salmon	9	1
Red w. pink	13	6
Light red	21	3
Pink w. orange	9	0
Pink	17	4

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.
[y]W. orange (or salmon, or pink) means that a tinge of orange (or salmon, or pink) color exists throughout the flesh, but the first color named is predominant.

Table 2-11. Distribution of numbers of fruits classified for flesh texture in fruits from a ms BC_3-F_2 to SSDL population segregating for male sterility[z].

	Male fertile	Male sterile
Meaty	28	12
Grainy	5	1
Gritty	3	-
Firm	12	3
Excellent	10	1
Crisp	21	12
Crisp and pulpy	4	-
Crisp and soft	25	5
Melt in your mouth	6	3
Meaty and stringy	1	-
Good	7	4
Fibrous	18	1
Crisp and chewy	15	7
Chewy	36	8
Soft and Chewy	18	2
Soft	21	3

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.

Table 2-12. Distribution of number of fruits classified for flavor in fruits from a ms BC_3-F_2 to SSDL population segregating for male sterility[z].

	Male fertile	Male sterile
Excellent	4	2
Good	59	19
Sweet	5	2
Clean	5	1
Fair	3	0
Weak	4	1
Average	10	2
OK	41	12
Poor	3	6
Cucumber/bitter	36	10
Bland	14	3
Fermented	16	13
Off	28	8

[z]The two populations were evaluated by the author and analyzed by their overall distribution by the Mann-Whitney test and found to be not significantly different.

CHAPTER 3
VIRUS RESISTANCE

Literature Review

Virus strain characterization and description

Watermelon Mosaic Virus (WMV) was first mentioned in the watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) literature in 1933 (Walker 1933). Subsequently, a distinction between two types of WMV was made based on symptoms (Anderson 1954). Some physical properties of WMV were identified as early as 1960 (Van Regenmortel 1971, Van Regenmortel et al. 1962), but it was not until 1965 that WMV-1 (PRSV-W) and WMV-2 were proposed to be different viruses based on serology and host range differences (Webb et al. 1965). A conflicting report stated that these were merely strains of the same virus based on serology, physical properties, vector relationships and symptoms in cucurbits (Milne & Grogan 1969). However, in 1979, it was confirmed that there are indeed two distinct viruses based on serology (Purcifull & Hiebert 1979).

Despite being unable to identify which watermelon mosaic virus was infecting a given crop in early reports, there were numerous reports from around the world that demonstrated how widespread this disease was and the extent of economic losses. In Florida, WMV-2 tends to predominate in the north, whereas WMV-1 is more prevalent in the south (Adlerz 1969, 1978a). In Southern California desert valleys, WMV-2 is more abundant than WMV-1 (Nameth et al. 1985). WMV-2 is found extensively in the irrigated southwestern and central Arizona melon fields (Nelson & Tuttle 1969). Other areas with serious infections causing significant loss are Georgia, Washington, New York and New Jersey (Clark & Adams 1977, Davis & Mizuki 1970, Provvidenti & Schoeder 1970). Worldwide reports from Cuba, Brazil, Hawaii, Japan, South Africa and Europe show the range and scope of the problem (De Sa & Kitajima 1991, Fischer & Lockhart 1974, Gerber 1969, Lindberg et al. 1956, Webb & Scott 1965, Yamamoto et al. 1982, Yamamoto 1986).

Viral strains and host range

In addition to the distinction made between WMV-1 and WMV-2, a number of strains of WMV-2 have been identified and examined. Three strains were reported in Hungary (Lovisolo 1980) and seven in India (Bhargava & Bhargava 1976). The

Indian strains reacted differently with various cucurbit cultivars. Some cultivars appeared resistant to all strains, whereas other cultivars were "symptomless carriers of one or more strains while being resistant to other strains" (Bhargava & Bhargava 1976).

The main feature that distinguishes WMV-2 from WMV-1 is the host range of WMV-2 (Webb & Scott 1965) which is greatly expanded beyond the cucurbit family (Toba 1962). In particular WMV-2 is known to infect 178 species from 27 families. Half of the susceptible species are legumes or cucurbits. Included in this list are hairy indigo, showy crotalaria, one leaf clover, sweet pea, Melothria pendula and Chenopodium amaranticolor (Inouye 1964, Purcifull et al. 1984). These weedy species represent a reservoir that can be a source of primary inoculum to initiate yearly epidemics (Adlerz 1974, 1978b). Although in some cases, the overwintering hosts are not known; hence, the primary source of inoculum is not known. This is pointed out by studies showing that aphid flights and infestation are not necessarily correlated with viral incidence (Adlerz 1974, Raccah 1986). Additionally, because aphids may travel many miles, it is possible that inoculum sources are adjacent or even close to commercial watermelon.

Virus infection symptoms, vectoring, and economic losses

The symptoms caused by WMV-2 in watermelon are mild mottling, blistering and malformation, reduced leaf size, marginal chlorosis, fruit shape abnormalities, reduced plant and fruit size, and reduced fruit yields (Anderson 1951, Andrus et al. 1971, Lindberg et al. 1956, Lovisolo 1980, Morton & Webb 1963, Purciful et al. 1988, Van Regenmortel 1971).

Quantitative measurements of yield loss in watermelon are few but significant. Demski & Chalkley (1972) found that in plants mechanically inoculated between the 1st true leaf and the runner stage, plant weight reduction was approximately 60% in three cultivars. The number of fruit harvested was reduced by 22-50% with an average of nearly 40% (Demski & Chalkley 1972). Total yield losses were 35-73% with an average of about 55%. Fruit size was reduced significantly in infected plants, and a higher percentage of abnormally shaped fruit were found in cultivars with elongated normal fruit (Demski & Chalkley 1972). In the same report, plants that were infected as late as 1st fruit set showed only a slight reduction in fruit set, but yield losses of 20% were observed. No data on the percent infection were given, and no distinction was made between

infected and uninfected plants within a plot when harvesting the fruit. No data on the sugar content of the fruit were given (Demski & Chalkley 1972).

More recently, an overall yield loss of 26% was reported in naturally infected plots that were 50% infected between 70-78 days after planting (Mora-Aguliera & Webb 1993). The spread of infection was monitored by sampling individual leaves every 3 days and analyzing them by ELISA. Final infection rates were 83-91% as determined by ELISA. Regression analysis showed that yield increase was directly proportional to delay of infection date at the rate of 0.146 kg/day. However, no correlation between time of infection and sugar content was found (Mora-Aguliera & Webb 1993). The vectors by which WMV-2 is spread in nature are various aphid species that transmit the virus in a non-persistent manner (Lopez-Abella et al. 1988, Powell 1991, Purcifull et al. 1984, Smith 1972, Toba 1963). To date, 15 species have been shown to vector the disease in Florida, with Aphis spiraecola, A. middletonii, A. gossypii, and Myzus persicae being the most important to viral epidemics (Adlerz 1974, 1978a). Forty two species are known to transmit WMV-2 worldwide (Castle 1992, Webb & Linda 1993, Yamamoto et al. 1982). WMV-2 has not been shown to be seed transmissible (Lecog et al. 1981, Purcifull et al. 1984).

Control of the virus with cultural management

Since plant resistance to the virus or its vectors is not commercially available, a variety of practices have been evaluated to avoid, delay, or reduce WMV-2 epidemics (Harpaz 1982, Hull & Davies 1992, Maelzer 1986). Host plants that can act as primary inoculum sources can be eliminated by cultivation or herbicides (Adlerz & Crall 1967). Reflective mulches such as aluminum foil reduce the number of aphids settling in the area, at least until the vines cover the mulch (Adlerz & Evertt 1968). Stylet oil can be sprayed on the crop, which serves to reduce transfer of the virus to the host and delays infection (Marco 1993).

Floating row covers have been used to exclude the vectors (Perring et al. 1989); however, the covers must be removed in order to allow pollination and fruit set. Additionally, trap crops such as wheat act as an alternate aphid food source, but not as a reproductive host, and are resistant to the virus (Toba et al. 1977). This reduces plant infection by reducing the number of viruliferous aphids (Toba 1962, 1963). Insecticides such as endosulfan have been applied with little benefit in reducing losses or infection rates (Webb & Linda 1993). These practices have not prevented epidemics, but as shown previously, every day

an epidemic is delayed represents increased benefits in terms of fruit yield and quality (Mora-Aguilera & Webb 1993). This also underscores the need to develop virus resistant cultivars.

The effects of ploidy level on watermelon trait expression

Triploid seedless watermelons were first developed in Japan (Kihara 1951). Triploid watermelon development and consumption has expanded rapidly in the past decade. There are numerous reports on the effects of polyploidy on various plant organs and disease resistance: 1) gigantism of leaves and flowers, 2) thicker leaves, rind, and seed coat, 3) increased seed size and chloroplast number, 4) decreased fruit size and seed set, 5) brittle vines, and 6) resistance to some pathogens (Kihara 1951, Green & Stevenson 1962, Shimotsuma 1965, Ho et al. 1990, Hopkins & Elmstrom 1993). Triploids are produced by using a tetraploid as the seed parent and a diploid as the pollen parent (Kihara 1951). Consequently, the tetraploid contributes two alleles to the triploid, whereas the diploid contributes one. The desirability of developing resistant cultivars has been established (Shimotsuma 1965). One area that has not been well reported is the inheritance of WMV-2 resistance and how

that mode of inheritance will affect the development of hybrid watermelon varieties, both diploid and triploid. There are several previous reports of polyploidy in cucurbits and its effect on gene expression or disease resistance. Green (1959) studied the effect of ploidy differences on horticultural characteristics and Fusarium wilt in watermelon. The horticultural characteristics were fruit shape and weight, hollowheart, soluble solids, seed coat size, the thickness, toughness and color of rind. Fruit shape is thought to be governed by a dominant gene for round fruit and a recessive gene for elongated fruit (Henderson 1977). However, additional genes may influence fruit shape. It was found that tetraploids derived from elongated diploids tend to be less elongated than the original diploid variety (Green & Stevenson 1962). Triploids from all combinations tested had round fruits.

Fruit of triploids from reciprocal crosses had significantly different weights, and fruits from triploid hybrids were smaller than diploid hybrids (Green & Stevenson 1962). Hollowheart was appreciably more severe in triploids than in either parent, and certain parental combinations were worse than others (Green & Stevenson 1962). Tetraploids had more hollowheart than the diploid line from which they were derived (Green & Stevenson 1962). Large fruited

triploid varieties had a higher incidence and severity of hollowheart (Green & Stevenson 1962). Sugar content as measured by soluble solids was slightly lower in triploids and tetraploids when compared to diploids (Green & Stevenson 1962). Additionally, there was an off-flavor associated with some tetraploids and to a lesser extent triploids (Green & Stevenson 1962). The rind thickness was greatest in triploids and least in diploids, with little to no reciprocal differences (Green & Stevenson 1962). Rind toughness was greatest in tetraploids, intermediate in triploids and least in diploids (Green & Stevenson 1962). Rind color is thought to be governed by a single gene with dark green dominant to light green (Poole 1944). In triploids where the tetraploid was light green and the diploid was dark green, the phenotype of the fruit was dark green. Fruit from reciprocal crosses were the same color indicating no dosage effect in triploids in this single gene trait. There was no ploidy effect on rind color because tetraploids derived from light or dark green diploids were not different in color (Green & Stevenson 1962). Seed coat size in triploid fruits was found to be strongly influenced by maternal seed size (Green & Stevenson 1962).

Green (1959) also studied the resistance to Fusarium wilt. It was noted that field resistant varieties were

often susceptible in greenhouse seedling tests. However, a good differential screening procedure was developed. The inheritance of the wilt resistant material used in this study was not clearly identified. One report stated that Fusarium resistance in watermelon was recessive and did not establish any clear F_2 ratios (Orton 1911). A subsequent report found resistance to be dominant, but no mention was made regarding the number of genes involved (Welch & Melhus 1942). Porter (1937a) found that Fusarium resistance in watermelon was controlled by at least two factors. Despite this lack of clarity, the wilt resistance of susceptible and resistant diploid, triploid and tetraploid watermelon lines was studied (Green 1959). As the ploidy of wilt resistant variety 'Klondike' increased, so did its susceptibility to the pathogen (Green 1959). Tetraploids of the wilt susceptible diploid varieties 'Earlybird' and 'Early Canada' were either more susceptible to wilt or equivalent to the diploid (Green 1959). Triploids derived from a "susceptible" tetraploid and a wilt resistant diploid were susceptible, as were triploids derived from susceptible x susceptible (Green 1959).

The conclusions reached by Green (1959) were that there was no increase in disease resistance due to ploidy. Susceptible x susceptible, susceptible x resistant and

resistant x resistant did not have adequate resistance. Resistant x susceptible was not tested. Both parents would need to be wilt resistant to produce a wilt resistant triploid (Green 1959).

Hopkins & Elmstrom (1993) have noted that triploid and tetraploid watermelons are less susceptible to bacterial fruit blotch even though no genetic resistance has been identified in the diploid material used to develop the tetraploids or triploid hybrids. No reason for this observation has been conclusively proven.

Gene dosage effects in watermelon and other cucurbits

Balgooyen (1972) examined triploid squash and found that single gene dominant traits were still expressed in the triploid even when the diploid male was the only parent expressing the trait. The disease resistance of triploid and tetraploid watermelons has been mentioned by several authors, but usually tetraploids and triploids were more resistant to Fusarium wilt than diploid watermelons (Kihara 1951). No data were presented to demonstrate this (Anderson 1951). Andrus et al.(1971) stated that watermelon polyploids seem to have an extra degree of field tolerance to unspecified disease when compared to diploids. He also stated that tetraploids with Fusarium wilt resistance had

been developed. However, no data to confirm this were presented (Andrus et al. 1971). Henderson (1977), using both resistant and susceptible cultivars demonstrated that tetraploids were more resistant to anthracnose than diploid parental lines. Anthracnose resistance is monogenic dominant (Winstead et al. 1959). Henderson also examined gene dosage effects in triploids and found that a single gene for resistance resulted in lower levels of disease incidence but that two resistant alleles were significantly better than one. Having all three alleles resistant was more effective than two, but the latter difference was not as great as the difference between one and two. Therefore, a definite gene dosage effect was found (Henderson 1977).

Gene dosage effects due to polyploidy in other species

Other examples of gene dosage effects (due to polyploidy) on the resistance of plants to pests or pathogens can be given. Vallejo et al. (1995) found a dosage dependent response to Potato Virus Y in tetraploid potato hybrids. It was proposed that homozygous plants were resistant and heterozygotes expressed a necrotic response. Although not all families fit the expected model, this was attributed to the necrotic reaction being dependent upon

temperature, viral strain, inoculum concentration and host genotype (Vallejo et al. 1995).

Novy & Helgeson (1994) fused protoplasts of resistant and susceptible potato plants and observed that the plants derived from these fusions were intermediate in resistance to PVY, and progeny retained this resistance when backcrossed to the susceptible parent. Susceptibility was based on ELISA results and plant reaction. Varieties with a susceptible reaction were characterized by stunting, chlorotic and necrotic leaves, and lower leaf drop. Plants with a resistant reaction had similar but less severe symptoms and no lower leaf drop (Novy & Helgeson 1994).

Busey found that polyploid clones of St. Augustine grass were more resistant to sting nematodes than a diploid clone. Resistance was evaluated based on numbers of nematodes per pot, shoot and root dry weight, and transpiration rates (Busey et al. 1993).

Plantains and bananas are triploid herbs derived from interspecific crosses. Resistance to black sigatoka, a fungal leaf spot disease, was studied (Ortiz & Vuylsteke 1994). Two susceptible triploid plantain cultivars were crossed with a resistant wild diploid banana, and progeny were evaluated for resistance by the following criteria: susceptible plants with less than 8 leaves without spots and

partially resistant as having 10 or more leaves without spots. Resistance was found to be governed by one major recessive allele and two independent alleles with additive effects. It was concluded that tetraploids exhibited a dosage effect for increased resistance (Ortiz & Vuylsteke 1994).

Materials and Methods

Developing and testing WMV-2 resistant lines

The breeding line with resistance to WMV-2 (Elisa) was developed at the University of Florida by Dr. J. Crall, Dr. G. Elmstrom and the author. In 1981, Dr. J. Crall crossed the WMV-2 and potentially ZYMV resistant line (Boyhan et al. 1992) 'Egun' with breeding lines of varying fruit types. The first backcross progenies were mechanically inoculated by Dr. W. Adlerz, and plants that did not show symptoms were presumed to be non-infected. Subsequent generations were tested for resistance by mechanically inoculating seedlings and evaluating the plants based on visual symptoms. A few plants in several generations showed mild symptoms. Additionally, two generations were field grown and tested for the presence of WMV-2 by ELISA. As with previous testing, some plants tested positive for the virus. In

subsequent field trials, whereas other varieties have shown obvious viral symptoms, the line used in this study has shown little to no visual symptoms. However, no firm conclusions concerning the resistance/susceptibility or inheritance of the trait could be reached due to the breeding procedures used. The breeding line used in this experiment has been termed 'Elisa'. This line is characterized by fruit with the following characteristics: elongated shape, 10-12 kgs., rind color of pale, solid light green, rind thickness 10-15 cm, regular red flesh, good texture, acceptable flavor, sugar content of 11%, and many small seeds. Phenotypically, the plants and fruit are uniform (Fig. 3-1).

A virus resistant tetraploid was developed from 'Elisa' by treating 7-day-old seedlings with colchicine according to previous methods (Kihara 1951). This line has been termed 'Elisa 4X'. The fruit of the tetraploid is reduced in length and seed number compared to the diploid. However these are well known associations of polyploidy in watermelon (Kihara 1951, Green & Stevenson 1962). 'Elisa 2X' and 'Elisa 4X' are considered resistant parents. The susceptible diploid parent used in this experiment was 'Calsweet'. The susceptible tetraploid was developed by treating 'Calsweet' seedlings with colchicine according to previous methods (Fig. 3-2).

Fig. 3-1. Watermelon line 'Elisa' tolerant to WMV-2.

Fig. 3-2. WMV-2 susceptible lines from top to bottom Calsweet 4x, Calsweet 4x X Calsweet, Calsweet. Note the effect of polyploidy, which tends to decrease fruit length.

Since the resistance had not been recently tested and has never been shown to be uniform, a preliminary greenhouse and field screening was conducted. Additionally, it was not known whether the resistance was race specific. To test for strain specificity and confirm resistance, 50 7-day-old seedings of each of the four parental lines (Elisa 2X, Elisa 4X, Calsweet, and Calsweet 4X) (Table 3-1) were mechanically inoculated with an isolate of WMV-2 obtained from Dr. D. Purcifull and another 50 each were mechanically inoculated with a strain from Dr. Susan Webb (Bos 1983).

Table 3-1. Parental varieties, ploidy level and plant disease reaction used in the viral resistance screening.

Variety	Ploidy level	Virus reaction
Elisa 2X	2X	Resistant
Elisa 4X	4X	Resistant
Calsweet	2X	Susceptible
Calsweet 4X	4X	Susceptible

The plants were evaluated visually for viral symptoms at first fruit set. Additionally, one single, fully expanded leaf close to the growing tip of each plant was removed and

used to test for the presence of WMV-2 by indirect ELISA using antisera from Agdia (Elkhart, Ind.) (Clark & Adams 1977).

In addition to testing one leaf per plant, single leaves were removed from five different runners of the diploid resistant line and each tested individually for WMV-2 by indirect ELISA.

Development of experimental hybrids

To determine whether ploidy level and gene dosage has any effect on the resistance to WMV-2, the four parental lines were used to make experimental hybrids. Fifty plants of each tetraploid parent and ten of each diploid parent were established via transplants. Reciprocal crosses were made with the diploid lines (Table 3-2)(Fig. 3-3).

Table 3-2. Diploid varieties used in the WMV-2 resistance experiment.

Variety	Relative resistance allele frequency
Elisa	2
Elisa X Calsweet	1
Calsweet X Elisa	1
Calsweet	0

In addition each diploid was crossed with each tetraploid in order to make all possible triploid combinations: Resistant X resistant, resistant X susceptible, susceptible X resistant and susceptible X susceptible (Table 3-3)(Fig. 3-4).

Table 3-3. Triploid varieties used in the WMV-2 resistance experiment.

Variety	Relative resistance allele frequency
Elisa 4X X Elisa 2X	3
Elisa 4X X Calsweet 2X	2
Calsweet 4X X Elisa 2X	1
Calsweet 4X X Calsweet 2X	0

The plants used for making crosses were not examined for their WMV-2 resistance. Mature fruit were collected, and the seeds were removed, cleaned and dried. The seeds of all the fruit representing a particular cross were bulked together.

Fig. 3-3. Diploid watermelon entries in the WMV-2 trial. From left to right Elisa, Elisa X Calsweet, Calsweet X Elisa, Calsweet.

Fig. 3-4. Triploid watermelon entries in the WMV-2 trial. From left to right Elisa 4x X Elisa 2x, Elisa 4x X Calsweet 2x, Calsweet 4x X Elisa 2x, Calsweet 4x X Calsweet 2x.

Viral inoculation and field trial protocols

To determine the effectiveness of this resistance and whether ploidy and gene dosage have any effect on this resistance, a field trial using the four parental lines (Table 3-1), the two diploid hybrids (Table 3-2) and the four triploid hybrids (Table 3-3) was carried out.

Seeds of each experimental line were planted in 72-cell trays using Pro-Mix 200 soil. The trays were kept in a greenhouse at ambient temperature. Initially the trays were watered every 2-3 days; however, when the seedlings reached the 2-3 leaf stage, they were watered everyday. The plants were fertilized with a 20-20-20 mix once per week.

One third of the seedlings of each variety were mechanically inoculated with WMV-2 at the cotyledon stage and a second time at the 2-3 leaf stage. The procedures for inoculation were standard (Bos 1983) except for the use of Scotch-Brite Pads used for inoculation pads. The WMV-2 isolate (FC-1656) was obtained from Dr. D. Purcifull and propagated in the pumpkin (Cucurbita pepo) variety 'Small Sugar Pumpkin'. Purity of the inoculum was checked by sending a leaf sample to Agdia (Elkhart, Ind.), and having it analyzed for the presence of other potyviruses that infect watermelon.

The field at the Gainesville Horticulture Research Unit was prepared by the University of Florida farm crew personnel. Rows were 2.44 m apart. Raised beds (15 cm high beds X .91 m wide) were fumigated with 448 kg/ha 98%:2% methyl bromide:chloropicram and covered with black plastic mulch. A total of 30 rows were established and the pattern was three rows alternating with a roadway. The preplant fertilizer used was 10-10-10 at 840 kg/ha. Fifty six kg of N and K (NH_4NO_3, KNO_3) were applied through the drip irrigation system using a peristaltic pump. Irrigation was initially once every other day for 30 min and increased to once per day for 60-90 min as the crop progressed. Insecticides (Asana 437 ml/ha, Dithane 3.4 kg/ha, and Lannate 2.34 l/ha) were sprayed once for aphids and once for cabbage looper caterpillars. Fungicides (Bravo 720 3.5 l/ha, Maneb 2.67 l/ha, Kocide 2.2 kg/ha, and Topsin .56 kg/ha) were applied once or twice per week. Herbicides were applied once preplant (Prefar 5.6 kg/ha and Alanap 4.5 kg/ha)and twice postplant (Roundup 1.1 kg ai/ha and Permit 46 g ai/ha) for purple nutsedge and grasses. These cultural practices were based on University of Florida crop recommendations and soil test results (Hochmuth & Elmstrom 1992).

Field trial treatments and leaf sampling procedures

The field trial consisted of the 10 varieties previously mentioned (Tables 3-1, 3-2, 3-3) with 3 treatments, 10 replicates per treatment and 5 plants per replicate. Two of the treatments were as follows: mechanically inoculated plants or non-inoculated plants, with both treatments covered by floating row covers to exclude insect vectors (Fig. 3-5). All row covers were removed at the time of first female flowering to allow for pollination to occur. The third treatment was non-inoculated plants without row covers.

The plants were visually evaluated for viral infection at first fruit set. Additionally, leaf samples were taken at first fruit set and first harvest for analysis by indirect ELISA. The first sampling consisted of one fully expanded leaf from a main runner on each plant in one non-inoculated, three open and four inoculated replicates. Each leaf was analyzed individually. For the second sampling, the procedure remained the same except that two non-inoculated, three open and two inoculated replicates were sampled. Only one non-inoculated and one open replicate were included in both samplings (fruit set and harvest). To determine if the virus was unevenly distributed in either the resistant or susceptible plants, single leaves were

removed from five different runners of five diploid resistant and susceptible parental plants and each tested individually for WMV-2 by indirect ELISA.

To determine the effects of resistance on fruit yield and quality, mature fruits were harvested for data collection. The data categories were: weight, size, soluble solids content (brix), flesh color, rind thickness, seed number and size, and hollowheart. The total fruit yield, weight and soluble solids content were the most important criteria in determining the resistance to WMV-2. The effect of the WMV-2 resistance was analyzed by comparing the three treatments for a given variety, but the comparison of inoculated vs. non-inoculated varieties was considered the most important as the third treatment served primarily to test the effect of row covers on the plants. The statistical method used for the infection rates was Tukeys. The statistical method used to test for non-random distribution of the virus in the plants was the permutation method of Fisher. The statistical method used to test for differences in total yield, fruit number, fruit weight, rind thickness, incidence and number of hard seeds and soluble solids (sugar content) was Duncans. The statistical method used to test for differences in the incidence and size of hollowheart was Fishers Exact Test. These procedures were recommended by

University of Florida Statistics Department personnel and were performed using SAS software.

Fig. 3-5. Row covers over plots containing experimental watermelon hybrids inoculated or non-inoculated with WMV-2. Plants which are not covered were the 'open' treatment which was non-inoculated.

Results and Discussion

ELISA data from the leaf sampling at first fruit set

After mechanically inoculating the plants, the ELISA data from tissue samples taken at first fruit set show that among diploids, the resistant line 'Elisa' exhibited some plants with a viral titer, but at a significantly lower rate (25%) of infection than the susceptible line 'Calsweet' (70%) (Table 3-4, 3-5). The reciprocal hybrids between these two parents became infected at an intermediate rate (50%)(Table 3-5). The tetraploid version of 'Elisa' also became infected at significantly lower rate (15%) than the tetraploid version of 'Calsweet'(85%) (Table 3-5). Among triploids there was no significant difference in the rate of infection which ranged from 55% to 75% (Table 3-5). It should be noted that the viral titer of the 'Elisa' plants, which were scored as positive for the virus, was generally lower than that of the 'Calsweet' plants (Table 3-4).

Visual evaluation of viral infection

The results of the visual evaluation for viral symptoms, that was made at first fruit set, corresponded fairly well to the ELISA data with the exception of the

triploids. In the triploids there appeared to be a dosage effect in the inoculated treatment with the susceptible X susceptible having the highest infection rate (95%), followed by the susceptible X resistant (30%), the resistant X susceptible (10%), and the resistant X resistant (5%) (Table 3-6). Percent infection can sometimes be estimated visually but not as accurately as ELISA data (Table 3-6, 3-7).

Variation in viral titer within the plant

In addition to testing for percent infection, the distribution of the virus within plants was studied. In the resistant line 'Elisa' the data show that there can be significant differences in the viral titer in leaves from different runners and that it is possible to pick a leaf from a runner with no viral titer and get a negative result that may not be indicative of the whole plant. There were a few, but insignificant, differences in the viral titer between runners in the susceptible variety 'Calsweet' (Table 3-8).

Elisa data from the leaves sampled at harvest time

When comparing the ELISA data from leaf samples taken from non-inoculated and open plants at harvest time (Table

3-7) to the data from inoculated plants sampled at first fruit set (Table 3-5), it seems that the relative percent infection observed in non-inoculated and open plants parallel the initial infection percentages obtained by mechanical inoculation. Once the covers were removed, the resistant tetraploid and triploid resistant x resistant non-inoculated plants were both significantly lower than the susceptible tetraploid and other triploids in the percent infection with WMV-2 acquired by natural vector spread of the virus (Table 3-7). There were no differences among the diploids (Table 3-7).

In the open treatment, the resistant tetraploid was significantly lower in percent infection than the susceptible tetraploid, and the triploid resistant x resistant was lower than other triploids (Table 3-7). Additionally, the resistant diploid and both reciprocal hybrids were significantly lower in viral infection rates than the susceptible diploid (Table 3-7).

At first fruit set, the open treatment had virtually no infection; hence, for purposes of statistical treatment, once the covers were removed from the non-inoculated treatment, the ELISA data from the two treatments could be combined to achieve more power. The results from the combination show the resistant diploid had significantly

less infection than the susceptible diploid, whereas the two diploid hybrids were intermediate in infection rates (Table 3-7). The resistant tetraploid and resistant x resistant triploid were both significantly lower in infection than the susceptible tetraploid, and the other three triploids (Table 3-7). These results suggest that tolerance brought about a delay of infection and possibly an inhibition of viral replication or viral movement.

The effect of virus inoculation on fruit yield and its components

Inoculation of tolerant diploid 'Elisa' plants did not reduce total yield below that of non-inoculated plants (Table 3-9). Inoculation of susceptible plants significantly reduced yield by 30% compared to non-inoculated plants (Table 3-9). The total yield loss of the reciprocal diploid hybrids was 15%, which was intermediate between the resistant and susceptible varieties but was not statistically different than either (Table 3-9).

The total yield of the tetraploid resistant and susceptible lines was similar to that of the diploids (Table 3-9). Inoculation of the resistant line did not result in reduced yield, whereas yield of the susceptible line was reduced by a statistically significant 27% (Table 3-9).

Among the triploid lines, the only line that didn't lose yield was the resistant x resistant. In contrast, yield

was reduced in the susceptible x susceptible plants by 35%, yield was reduced in the susceptible x resistant plants by 22%, and yield was reduced in the resistant x susceptible plants by 33%. The yield reductions of 35% and 33% were statistically significant, whereas the 22% yield reduction was not significant (Table 3-9).

The effects of mechanical inoculation on number of fruit set were minimal. The diploid susceptible line (Calsweet) had a significant reduction of fruit set per plot as did the triploid susceptible x susceptible line. The tetraploid susceptible line had a non-significant loss of fruit set. All other lines were unaffected in fruit set per plot (Table 3-10).

The fruit weight of all varieties were not significantly affected by inoculation with WMV-2. It should be noted that the fruit size was generally lower than expected (Table 3-11).

The effect of virus inoculation on fruit quality characteristics

The rind thickness of almost all varieties was unaffected by inoculation with WMV-2. The only variety that was significantly affected was the diploid resistant line 'Elisa' in which the rind thickness was thinner in fruits from the inoculated plants (Table 3-12).

Differences in the percentage of fruits with hard seeds

and number of seeds per fruit were not statistically significant between inoculated and non-inoculated plants in any of the four triploids (Table 3-13).

The incidence of hollowheart in fruits was statistically lower in three of the ten varieties that were inoculated with WMV-2, viz., the resistant diploid (Calsweet), the resistant tetraploid (Elisa 4X) and the resistant x susceptible triploid. The remaining seven varieties had no significant difference between fruits from the inoculated and non-inoculated plants (Table 3-14). There was no discernable or consistent pattern as to which varieties were affected by viral inoculation. As with hollowheart incidence, the size of the hollowheart in fruits was unaffected in 8 of the 10 varieties. The non-inoculated diploid susceptible line (Calsweet) had a larger hollowheart size than the inoculated plants. Fruit of the diploid resistant line (Elisa 2X) had a larger size hollowheart in the inoculated plants when compared to the non-inoculated plants. Both of these results are somewhat suspect due to the low number of melons with hollowheart used to make these comparisons (Table 3-14).

The other major component of interest to both consumer and farmers is the sugar content of the fruit. The sugar content of 9 out of 10 lines was not significantly affected

when comparing fruits from the inoculated and non-inoculated plants. The lone exception was the susceptible tetraploid (Calsweet 4x), in which inoculation with WMV-2 reduced sugar content (Table 3-15).

The flesh color distributions of the majority of the varieties were not significantly different when comparing fruits from the inoculated and non-inoculated plots. Of the three varieties that were different in their distribution, the direction of color change was inconsistent with respect to darker or lighter shades of color. Of these three varieties, 'Elisa 2X' x 'Calsweet 2X' and 'Calsweet 4X' were adversely affected (Appendix). The inoculated treatment had fewer fruits in the deeper red shades when compared to non-inoculated. The triploid variety 'Elisa 4X' x 'Elisa 2X' showed a movement from salmon and light red with orange in the non-inoculated fruit to medium red with orange in the inoculated plot. Flesh color was evaluated by placing fruits into the closest color category as judged visually. The Royal Horticultural Society Charts or colorimetry were not used.

Current findings compared to previous findings

In agreement with previous findings of Mora-Aguilera & Webb (1993), the soluble solids (sugar) content of both the

virus susceptible varieties was not significantly affected by WMV-2 infection. Similarly, the sugar content of the tolerant lines was not affected by WMV-2 infection.

The effects of polyploidy on virus reaction of watermelon plants were similar to Green's (1959) findings that tetraploids of Fusarium wilt susceptible varieties were either slightly more susceptible to the pathogen, or equivalent to the diploid in disease reaction. This is in agreement with Green's (1959) findings that there was no increase in disease resistance due to ploidy level. This is true of the WMV-2 reaction in which the WMV-2 infected susceptible tetraploid line was reduced in yield by 27%, whereas the WMV-2 infected susceptible diploid line was reduced by 30%. The tetraploid susceptible line did show a slightly higher initial infection than the susceptible diploid line. Also, the susceptible tetraploid fruit appeared to be a lighter shade of pink when infected with WMV-2, whereas the diploid was not affected as much.

The effect of polyploidy on virus reaction of tolerant plants was different from that reported by Green (1959), who found that tetraploids derived from wilt resistant diploids were more susceptible to wilt than the diploids. The tetraploid WMV-2 tolerant plants exhibited a lower WMV-2

infection than the diploid plants (Table 3-7). However, the triploid lines had a higher infection than the diploids (Table 3-5). Neither the tetraploid nor the diploid had any yield or quality loss when challenged with WMV-2.

The effects of gene dosage on virus reaction were similar to the findings of Green (1959) that triploid watermelons derived from a susceptible tetraploid and a resistant diploid were susceptible and that tolerant x tolerant became infected with WMV-2 infected at a rate not different from the susceptible line when mechanically inoculated. However, there were distinct differences from Green's (1959) findings, i.e., the rate of natural infection in the tolerant x tolerant was lower than in the susceptible line. Additionally, the tolerant x tolerant did not have a reduction in yield when challenged with WMV-2, whereas in Green's (1959) study, the resistant x resistant did not have adequate resistance. There were no other gene dosage effects on any of the characters measured in this study.

The effects of gene dosage on virus reaction were different from Henderson's (1977) study that found a gene dosage effect for anthracnose resistance in triploid watermelons. In the present study, no gene dosage effect was found for percent infection, fruit yield, or any of the quality aspects of watermelon (Tables 3-9 through 3-15).

The present study found no gene dosage effect for WMV-2 resistance in watermelons using these lines. This finding is different from Vallejo et al. (1995) who found a gene dosage effect for PVY virus resistance in tetraploid potatoes. A summary of the results is given below:

- Due to a low infection rate in the resistant lines the resistance of these breeding lines should be considered as tolerance.
- The resistant plants that do become infected generally have a lower viral titer than susceptible lines.
- Among the resistant plants which do become infected, there can be significant differences between runners in viral concentration.
- Visual evaluation usually correlates well with ELISA data.
- In uncovered plots, WMV-2 resistant lines seem to delay the onset of infection.
- The total yield, fruit number, and fruit size of the resistant lines were not affected by mechanical inoculation with WMV-2 virus.
- The total yield and fruit number of the susceptible lines were generally reduced by WMV-2 infection produced by mechanical inoculation.
- Diploid hybrids between resistant and susceptible lines were intermediate in yield.
- The only triploid hybrid in which virus inoculation did not cause a reduction in yield was the resistant x resistant line.
- In order to release resistant diploid and triploid hybrids, both parents will need to be resistant.

- For both the tolerant and susceptible lines, the fruit characteristics sugar content, incidence and severity of hollowheart, number of hard seeds, and rind thickness were generally unaffected by WMV-2 inoculation.

Table 3-4. Indirect ELISA values of diploid susceptible and tolerant watermelon lines mechanically inoculated with WMV-2

Plant no.	Calsweet 2x (susceptible)	Elisa 2x (tolerant)
1	3.523	0.195
2	3.738	0.105
3	3.585	0.129
4	3.540	0.099
5	3.392	2.536
6	3.188	0.136
7	3.519	2.910
8	3.449	0.367
9	3.003	0.258
10	2.509	0.979

Note: The positive control Elisa value was 3.551 and the negative control value was 0.222.

Table 3-5. ELISA evaluation of WMV-2 percent infection rates in experimental watermelon hybrids at first fruit set.[z]

Variety or F_1 hybrid	Ploidy level	Inoculated[y] with row cover	Non-inoculated with row cover[x]	Non-inoculated without cover (Open[w])
Calsweet 2x[v]	2X	70 b[t]	0 a	7 a
Elisa 2x[u]	2X	25 a	0 a	0 a
Calsweet 2x X Elisa 2x	2X	50 ab	0 a	0 a
Elisa 2x X Calsweet 2x	2X	50 ab	0 a	0 a
Elisa 4x	4X	15 a	0 a	7 a
Calsweet 4x	4X	85 b	0 a	0 a
Calsweet 4x X Calsweet 2x	3X	75 a	0 a	0 a
Calsweet 4x X Elisa 2x	3X	55 a	0 a	0 a
Elisa 4x X Calsweet 2x	3X	75 a	0 a	15 a
Elisa 4x X Elisa 2x	3X	60 a	0 a	0 a

[z]One leaf per plant was sampled and analyzed individually.

[y]Sample size per entry was 20 plants.

[x]Sample size per entry was 10 plants.

[w]Sample size per entry was 15 plants.

[v]WMV-2 susceptible line.

[u]WMV-2 tolerant line.

[t]Within a ploidy level, varieties with letters in common are not significantly different at a .05 level according to the Tukey test.

Table 3-6. Visual evaluation at first fruit set of watermelon plants mechanically inoculated with WMV-2.

Variety or F_1 hybrid	% plants infected[z]
Calsweet 2x	90
Elisa 2x	25
Calsweet 2x X Elisa 2x	85
Elisa 2x X Calsweet 2x	50
Elisa 4x	10
Calsweet 4x	90
Calsweet 4x X Calsweet 2x	95
Calsweet 4x X Elisa 2x	30
Elisa 4x X Calsweet 2x	10
Elisa 4x X Elisa 2x	5

[z]A plant was considered infected if any portion of the plant showed traditional symptoms of viral infection, i.e., mottle.

Table 3-7. ELISA determined WMV-2 percent infection in experimental watermelon hybrids at harvest.[z]

Variety or F_1 hybrid	Ploidy level	Inoculated with row covers[y]	Non-inoculated with row covers[x]	Non-inoculated without row covers (Open)[w]	Non-inoculated + Open
Calsweet 2x	2X	100 a[v]	50 a	94 a	76 a
Elisa 2x	2X	30 b	30 a	27 b	28 b
Calsweet 2x X Elisa 2x	2X	70 ab	80 a	15 b	40 ab
Elisa 2x X Calsweet 2x	2X	70 ab	50 a	40 b	44 ab
Elisa 4x	4X	20 b	10 b	7 b	8 b
Calsweet 4x	4X	100 a	80 a	53 a	64 a
Calsweet 4x X Calsweet 2x	3X	100 a	90 a	60 a	72 a
Calsweet 4x X Elisa 2x	3X	80 a	100 a	53 a	72 a
Elisa 4x X Calsweet 2x	3X	60 a	80 a	60 a	68 a
Elisa 4x X Elisa 2x	3X	30 b	10 b	15 b	12 b

[z]One leaf per plant was sampled and analyzed individually.

[y]Sample size was 10 plants per entry.

[x]Sample size was 10 plants per entry.

[w]Sample size was 15 plants per entry; plants non-inoculated and non covered.

[y]Within a ploidy level, varieties with letters in common are not significantly different at a .05 level according to the Tukey test.

Table 3-8. Localization of WMV-2 infection at first fruit set in mechanically inoculated watermelon plants.

Calsweet 2x (susceptible)					
Plant no.	Leaf				
	A	B	C	D	E
1	9[z]	4	10	10	0
2	10	6	10	10	9
3	10	10	10	6	10
4	8	9	9	9	9
5[y]	10	9	10	7	9

Elisa 2x (resistant)					
Plant no.	Leaf				
	A	B	C	D	E
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4[x]	8	6	1	1	2
5	1	1	0	0	2

Note: Five leaves per plant, each individually analyzed by ELISA.
[z]Leaf scored from 0 to 10 where 0= ELISA<0.2, 1=~0.4, 3=~1.0, 4=~1.4, 6=~1.9, 8=~2.4, 9=~2.8, and 10= >3.0.
[y]Within the susceptible variety, the plants were not significantly different at the .05 level according to the permutation method of Fisher.
[x]Within the tolerant line, the plants were significantly different at the .05 level according to the permutation method of Fisher.

Table 3-9. Effect of WMV-2 infection on total yield of experimental watermelon hybrids (kg/plot).

Variety or F_1 hybrid	Inoculated with row cover	Non-inoculated with row cover	Open[z]
Calsweet 2x	33.0 b[y]	47.4 a	44.2 ab
Elisa 2x	61.4 a	43.3 b	39.7 b
Calsweet 2x X Elisa 2x	49.5 b	56.4 ab	62.7 a
Elisa 2x X Calsweet 2x	43.2 a	49.2 a	47.7 a
Elisa 4x	33.4 b	32.1 b	41.2 a
Calsweet 4x	19.0 c	26.8 b	34.2 a
Calsweet 4x X Calsweet 2x	26.7 b	41.5 a	46.0 a
Calsweet 4x X Elisa 2x	33.0 a	42.2 a	39.9 a
Elisa 4x X Calsweet 2x	84.2 b	118.6 a	112.9 a
Elisa 4x X Elisa 2x	128.5 a	113.1 a	124.6 a

[z]Plants were non-inoculated and without row covers.

[y]Within a variety, letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 3-10. Effect of WMV-2 infection on mature fruit harvested per plot of experimental watermelon hybrids.

Variety or F_1 hybrid	Non-inoculated with row covers	Inoculated with row covers	Open[z]
Calsweet 2x	8.2a[y]	6.9b	5.9b
Calsweet 2x X Elisa 2x	10.8a	10.1a	10.9a
Elisa 2x X Calsweet 2x	9.8a	9.2a	9.9a
Elisa 2x	15.0b	18.4a	11.8b
Calsweet 4x	6.1a	5.1a	6.4a
Elisa 4x	10.9a	12.5a	13.0a
Calsweet 4x X Calsweet 2x	9.5a	6.4b	8.4ab
Calsweet 4x X Elisa 2x	8.5a	7.2a	8.1a
Elisa 4x X Calsweet 2x	12.1a	9.4a	11.5a
Elisa 4x X Elisa 2x	15.4a	17.7a	16.5a

[z]Plants were non-inoculated and non-covered.

[y]Within a variety, letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 3-11. Effect of WMV-2 infection on fruit weight (kg.) of experimental watermelon hybrids.

Variety or F_1 hybrid	Non-inoculated with row covers	Inoculated with row covers	Open[z]
Calsweet 2x	5.7b[y]	5.1b	7.5a
Calsweet 2x X Elisa 2x	5.2a	4.9a	5.7a
Elisa 2x X Calsweet 2x	5.0a	4.7a	4.8a
Elisa 2x	3.1a	3.3a	3.4a
Calsweet 4x	4.4b	3.7b	5.4a
Elisa 4x	3.0a	2.8a	3.2a
Calsweet 4x X Calsweet 2x	4.8ab	4.4b	5.5a
Calsweet 4x X Elisa 2x	4.9a	4.6a	4.9a
Elisa 4x X Calsweet 2x	4.4a	4.1a	4.4a
Elisa 4x X Elisa 2x	3.3a	3.3a	3.44a

[z]Plants were non-inoculated and without row covers.

[y]Within a variety, letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 3-12. Effect of WMV-2 infection on the rind thickness (mm) of experimental watermelon hybrids.

Variety or F_1 hybrid	Non-inoculated with row covers	Inoculated with row covers	Open[z]
Calsweet 2x	12.25 a[y]	11.58 a	12.98 a
Calsweet 2x X Elisa 2x	11.98 a	11.67 a	12.03 a
Elisa 2x X Calsweet 2x	12.46 a	11.88 a	11.72 a
Elisa 2x	13.0 a	11.2 b	11.69 ab
Calsweet 4x	17.65 a	17.65 a	15.99 a
Elisa 4x	14.34 a	13.44 a	13.07 a
Calsweet 4x X Calsweet 2x	15.66 a	15.41 a	14.43 a
Calsweet 4x X Elisa 2x	17.55 a	15.28 a	15.51 a
Elisa 4x X Calsweet 2x	15.15 a	14.09 a	13.70 a
Elisa 4x X Elisa 2x	13.41 a	13.41 a	12.88 a

[z]Plants were non-inoculated and without row covers.

[y]Within a variety, letters in common indicate no significant differences at the .05 level according to Duncans new multiple range test.

Table 3-13. Effect of WMV-2 infection on the incidence and number of hard seeds in triploid watermelons.

F_1 hybrid	Non-Inoculated with row covers		Inoculated with row covers		Open[z]	
	% fruit with seeds	No. of seeds/ melon	% fruit with seeds	No. of seeds/ melon	% fruit with seeds	No. of seeds/ melon
Calsweet 4x X Calsweet 2x	58 a[y]	1.90 a	43 a	1.85 a	60 a	2.21 a
Calsweet 4x X Elisa 2x	49 a	2.00 a	44 a	1.50 a	56 a	2.10 a
Elisa 4x X Calsweet 2x	54 a	1.95 a	53 a	2.00 a	52 a	1.35 a
Elisa 4x X Elisa 2x	51 a	1.47 a	45 a	1.55 a	45 a	1.28 a

[z]Plants were non-inoculated and without row covers.

[y]Within a variety, letters in common indicate no significant difference at the .05 level according to Duncans new multiple range test.

Table 3-14. Effect of WMV-2 infection on the incidence and severity of hollowheart[z] (HH) in experimental watermelon hybrids.

	Non-inoculated with row covers		Inoculated with row covers		Open[y]	
Variety or F_1 hybrid	% fruit w/ HH	Ave. HH size (mm)	% fruit w/ HH	Ave. HH size (mm)	% fruit w/ HH	Ave. HH size (mm)
Calsweet 2x	7 b[x]	6 b	0 a	0 a	8 b	16 b
Calsweet 2x X Elisa 2x	14 a	10 a	7 a	10 a	9 a	11 a
Elisa 2x X Calsweet 2x	13 b	10 ab	4 ab	4 a	1 a	15 b
Elisa 2x	5 a	6 a	1 a	20 b	6 a	8 a
Calsweet 4x	14 a	16 a	7 a	6 a	13 a	16 a
Elisa 4x	20 b	15 a	5 a	17 a	9 a	10 a
Calsweet 4x X Calsweet 2x	36 b	20 a	33 ab	18 a	20 a	13 a
Calsweet 4x X Elisa 2x	21 b	11 a	26 b	8 a	9 a	13 a
Elisa 4x X Calsweet 2x	30 b	8 a	13 a	9 a	11 a	10 a
Elisa 4x X Elisa 2x	21 a	10 a	20 a	6 a	30 a	6 a

[z]Hollowheart is defined as a gap in the flesh of the melon.

[y]Plants were non-inoculated and without row covers.

[x]Within a variety, letters in common indicate no significant difference at the .05 level according to Fishers exact test.

Table 3-15. Effect of WMV-2 infection on soluble solids[z] (sugar) content of experimental watermelon hybrids.

Variety or F_1 hybrid	Non-inoculated with row covers	Inoculated with row covers	Open[y]
Calsweet 2x	8.73 b[x]	8.80 b	9.84 a
Calsweet 2x X Elisa 2x	9.78 a	9.48 a	9.79 a
Elisa 2x X Calsweet 2x	9.84 a	9.86 a	9.78 a
Elisa 2x	8.62 b	9.14 ab	9.34 a
Calsweet 4x	9.89 a	9.33 b	10.18 a
Elisa 4x	9.42 a	9.14 a	9.45 a
Calsweet 4x X Calsweet 2x	10.17 a	9.95 a	10.17 a
Calsweet 4x X Elisa 2x	10.61 a	10.34 a	10.34 a
Elisa 4x X Calsweet 2x	10.41 a	10.31 a	10.36 a
Elisa 4x X Elisa 2x	9.64 a	9.76 a	9.82 a

[z]All values determined by a hand held refractometer.

[y]Plants were non-inoculated and without row covers.

[z]Within a variety, letters in common indicate no significant differences at the .05 level according to Duncans new multiple range test.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Triploid Seed Production with Male-sterile Tetraploids

A diploid watermelon breeding line segregating for male sterility was treated with colchicine to produce a tetraploid male-sterile breeding line. This tetraploid male-sterile breeding line was used as a donor parent and crossed with a tetraploid breeding line SP90-1. A backcross breeding program was used to develop a BC_3-F_2 male-sterile tetraploid phenotypically similar to SP90-1.

The BC_3-F_2 male-sterile tetraploid SP90-1 and the original SP90-1 were both crossed with the diploid line 'SSDL' to produce the varieties 'Rubylee' and 'Flordalee', respectively. The two triploids were observed for the following characteristics: fruit number, fruit size, early and total yield, percent soluble solids, flesh color, texture and taste, hollowheart, rind thickness, rind necrosis and seed number.

The triploid 'Rubylee' was equivalent to 'Flordalee' in the important characteristics of fruit number, size and

yield. Additionally, 'Rubylee' was equivalent to 'Flordalee' in the other characteristics of seed number, rind thickness and rind necrosis. 'Rubylee' was inferior to 'Flordalee' with respect to percent soluble solids, flesh color, texture, taste and hollowheart.

To determine if male sterility is linked to percent soluble solids, flesh color, or hollowheart, two diploid BC_3-F_2 populations segregating for male sterility were grown. Fruit were harvested and classified based on their origin from male-sterile or male-fertile plants. The fruits were evaluated for percent soluble solids, flesh color, taste, and hollowheart. In both diploid populations, the male-sterile fruit were not statistically different from the male-fertile fruit for all characteristics examined. Therefore, it was concluded that male sterility was not significantly linked to percent soluble solids, flesh color, taste or hollowheart. If male sterility is indeed unlinked to these characteristics, additional backcrosses and selection should produce a male-sterile tetraploid that is functionally equivalent to SP90-1.

This is the first demonstrated employment of a tetraploid male-sterile watermelon for production of a triploid watermelon variety. The development of tetraploid male-sterile watermelon lines should eliminate the need for

a phenotypic marker system to distinguish triploids from tetraploids. This should expand the germplasm base that can be used to develop triploid combinations. If proper roguing is performed in the triploid seed production field, no tetraploid seed will be mixed in with the triploid seed that is produced. The use of male sterility should eliminate the need for hand pollination to produce hybrid seed and thereby lower the cost of seed production. If seed companies can keep their lines proprietary, it should stimulate additional investment in the development of new watermelon varieties.

A summary of the results, conclusions, and benefits is given below.

- Male sterility has been introduced into a tetraploid watermelon line via the backcross method.
- This tetraploid was used to produce a triploid that was equivalent to the variety 'Flordalee' with respect to fruit size, fruit number, total yield, incidence and number of hard seeds per melon, rind thickness, and rind necrosis.
- Further backcrosses should improve the performance of the tetraploid with respect to sugar content, flesh color, flesh texture, hollowheart, and flavor.
- Male-sterile plants can be used to produce 100% hybrid seed without the need for hand pollination.
- The cost of seed production should be lower.
- Germplasm may be kept proprietary.
- These factors should stimulate additional investment by seed companies and increase the profitability of

farmers.

- No field space will be wasted on tetraploids when producing triploid fruit.
- The germplasm base that can be used to produce triploid hybrids will be expanded by eliminating the need for a phenotypic marker system.
- Consumers will not be disappointed by purchasing a seeded melon after having paid for a seedless melon.

Watermelon Mosaic Virus-2 Resistance

A diploid watermelon breeding line with tolerance to WMV-2 was developed by Dr. J. Crall and Dr. G. Elmstrom. Seedlings of this virus tolerant line (Elisa 2X) were treated with colchicine, and the resulting tetraploid plants were identified by the author. Plants of the diploid and tetraploid virus tolerant lines were established in the field along with plants of the virus susceptible diploid variety 'Calsweet' and tetraploid 'Calsweet 4X'. Reciprocal crosses of the diploid lines were made, and all possible triploid combinations were made. Subsequently, a field trial using the four parent lines, the two diploid hybrids, and the four triploid hybrids was divided into three treatments: 1) WMV-2 mechanically inoculated plants protected from virus vectors with row covers, 2) non-inoculated plants protected from virus vectors with row covers, 3) non-inoculated, without row covers (open). All treatments were observed for

the following characteristics: fruit number, fruit size, total yield, percent soluble solids, flesh color, flesh texture and taste, hollowheart, rind thickness, rind necrosis, and seed number in triploids.

The WMV-2 infection achieved in the mechanically inoculated diploid, triploid,and tetraploid susceptible plants were 70, 75 and 90%, respectively, when measured by ELISA at first fruit set and 100% in all susceptible plants when measured at harvest time. The WMV-2 infection in the mechanically inoculated diploid, triploid and tetraploid tolerant plants were 20, 60, and 15%, respectively, at first fruit set and 30, 20, and 30% when measured at harvest time. This shows that the tolerant line does get infected and that the infection spreads within the plant. However, the overall infection is lower in the tolerant line. It was also found that the viral titer is lower in the tolerant line. Additionally, the virus was not uniformly distributed in the tolerant line.

The infection in the mechanically inoculated diploid F_1 hybrid plants was 50% at first fruit set and 70% at harvest. This was intermediate with respect to the parental lines. The infection in the mechanically inoculated triploid F_1 hybrids was as follows: a) the triploid plants with the female parent susceptible and the male parent tolerant was

55% at first fruit set and 100% at harvest, b) the triploid with the female parent tolerant and the male parent susceptible was 75% at first fruit set and 60% at harvest, and c) these levels were not significantly different from the triploid with both parents susceptible to the virus.

Since the infection level in the open treatment was negligible at first fruit set, the harvest time infection levels of the open and non-inoculated treatments were combined. The natural infection observed in this group of plants was comparable to the level obtained by mechanical inoculation observed at first fruit set.

The visual evaluation for percent infection observed at first fruit set correlated fairly well with the ELISA data with the exception of the triploid hybrids in which a dosage dependent response was observed by eye but was not borne out by the ELISA data. This could be either experimental bias or difficulty in classifying the more vigorous triploid vines.

For the WMV-2 inoculated plots with virus susceptible diploid, triploid, and tetraploid plants, the total yield was reduced by 30-35% when compared to non-inoculated plots. For the WMV-2 inoculated plots with virus tolerant diploid, triploid, and tetraploid plants, total yield did not decrease when compared to non-inoculated plots. The diploid hybrids between the tolerant and susceptible lines had a

total yield reduction of 13% and 18% when comparing inoculated and non-inoculated plots. This yield reduction was intermediate between the parental lines. The inoculated plots of the triploid with the female parent susceptible and the male parent tolerant had a total yield reduction of 23% when compared to the non-inoculated plots. The inoculated plots of the triploid with the female parent tolerant and the male parent susceptible had a total yield reduction of 29% when compared to the non-inoculated plots. The total yield reduction of these two triploid hybrids was much closer to the 36% yield reduction of the susceptible triploid than the tolerant triploid which had no total yield reduction loss. The conclusion drawn from this experiment is that to have either a seeded or seedless hybrid that does have reduced yield when challenged with WMV-2, both parents must be tolerant. Alternatively, an open-pollinated line with WMV-2 tolerance should not have a yield reduction either. Other genetic sources of WMV-2 resistance/tolerance, be they either transgenic or traditional, may not produce the same results.

The reduction in total yield was due to non-significant reductions in fruit weight in combination with significant reductions in fruit number in the diploid and triploid virus

susceptible lines. The reduction in fruit number in the tetraploid susceptible line was not significant.

The effects of mechanically inoculated WMV-2 infection on percent soluble solids, taste, texture, hollowheart size, rind thickness, rind necrosis, and the incidence and number of seeds in triploid fruit were not significantly different when comparing inoculated to non-inoculated plots with row covers. In contrast, the inoculation produced significantly less hollowheart in three of the ten WMV-2 inoculated lines (Calsweet 2X, Elisa 4X, and Elisa 4X x Calsweet 2X). Similarly, the flesh color of the WMV-2 susceptible lines seemed to be less red and more pink when infected with WMV-2.

A summary of the results, conclusions, and benefits is given below.

- Due to a low (but real) infection rate in the resistant lines the resistance of these breeding lines should be considered as tolerance.
- The tolerant plants that do become infected generally have a lower viral titer than susceptible lines.
- Among the tolerant plants which do become infected, there can be significant differences in viral concentration between runners on the same plant.
- Visual evaluation correlates well with ELISA data.
- In uncovered plots, tolerant lines seem to delay the onset of WMV-2 infection.

- The total yield, fruit number, and fruit size of the tolerant lines are not affected by mechanical inoculation with WMV-2 virus.
- The total yield and fruit number of the susceptible lines were generally reduced by WMV-2 infection produced by mechanical inoculation.
- Diploid hybrids between tolerant and susceptible lines were intermediate in yield compared with the parental yields.
- The only triploid hybrid that did not have a yield reduction was the tolerant x tolerant line.
- In order to release tolerant diploid and triploid hybrids, both parents will need to be tolerant.
- For both tolerant and susceptible lines, the fruit characteristics sugar content, incidence and severity of hollowheart, number of hard seeds, and rind thickness of all lines tested were generally unaffected by inoculation with WMV-2.

LIST OF REFERENCES

Abd-El-Hafez, A. A. 1981. Inheritance of flesh color, seed coat cracks and total soluble solids in watermelon and their genetic relationships. Acta Agron. Hung. 30:82-86.

Abd-El-Hafez, A. A. 1983. Types of dominance of fruit characters in various F1 hybrids in watermelon. Acta Agron. Hung. 32:107-114.

Abd-El-Hafez, A. A. 1985. Inheritance of flesh color, seed coat cracks and total soluble solids in watermelon and their genetic relations. II Quantitative characters and association between various characters. Acta Agron. Hung. 34(1-2):84-89.

Adlerz, W. C. 1969. Distribution of watermelon mosaic virus 1 and 2 in Florida. Proc. Fla. State Hortic. Soc. 81:161-165.

Adlerz, W. C. 1974. Spring aphid flight and incidence of watermelon mosaic virus. Phytopathology 64:350-353.

Adlerz, W. C. 1978a. Secondary spread of watermelon mosaic virus by Anuraphis middletonii. J. Econ. Entomol. 71:531-533.

Adlerz, W. C. 1978b. Watermelon mosaic virus 2 epidemics in Florida 1967-1977. J. Econ. Entomol. 71:596-597.

Adlerz, W. C., & J. M. Crall. 1967. Epidemiology and control of watermelon mosaic virus. Fla. Agr. Expt. Sta. Ann. Rep. 403.

Adlerz, W. C. & P. H. Evert 1968. Aluminum foil and white polyethylene mulches to repel aphids and control watermelon mosaic virus. J. Econ. Entomol. 61:1276-1279.

Allred, A. J. & G. Lucier. 1990. The U.S. watermelon industry. Report No. 9015. USDA, Washington D.C.

Anderson, C. W. 1951. Some preliminary observations on cucurbit viruses in Florida. Plant Dis. Rptr. 35:233-235.

Anderson, C. W. 1952. The distribution of cucurbit viruses in central Florida. Plant Dis. Rptr. 36:377-379.

Anderson, C. W. 1954. Two watermelon mosaic virus strains from central Florida. Phytopathology 44:198-202.

Andrus, C. F., V. S. Seshadri & P. C. Grimball. 1971. Production of seedless watermelons. Technical Bulletin No. 1425. USDA, Washington D.C.

Antignus, Y., B. Raccah, A. Galon & S. Cohen. 1989. Biological and serological characterization of zucchini yellow mosaic and watermelon mosaic virus-2 isolates in Israel. Phytoparasitica. 17:289-298.

Balgooyen, B. 1972. Expression of traits in triploids of Cucurbita moschata. PhD Thesis, Cornell Univ., Ithaca, N.Y.

Barham, W. S. 1956. A study of the Royal Golden watermelon with emphasis on the chlorotic characteristic of this variety. Proc. Amer. Soc. Hort. Sci. 67:487-489.

Barna, B. 1965. Evaluation of F1 hybrids of watermelon Kertesz. Szolezz. Foiskol. Kozl. 28(1):100-111.

Bhargava, B.,& K. S. Bhargava. 1976. Reactions of some cucurbit cultivars to seven strains of watermelon mosaic virus. Indian Phytopathol. 29:446-447.

Bos, L. 1983. Introduction to Plant Virology. Longman Press, Inc., New York, NY. 160 pp.

Boyhan G., J. D. Norton, B. J. Jacobsen & B. R. Abrahams. 1992. Evaluation of watermelon and related germ plasm for resistance to zucchini yellow mosaic. Plant Dis. 76:251-252.

Brar, J. S. & K. S. Nandpuri. 1974. Inheritance of total soluble solids, fruit shape, yield and its components in watermelon. Proc. 19th International Horticultural Congress I. Section 7, Vegetables, p. 711.

Brar, J. S. & K. S. Nandpuri. 1977. A note on the inheritance of total soluble solids in watermelon (Citrullus lanatus (Thunb.) Mansf.). Haryana J. Hort. Sci. 6:193-195.

Busey, P., R. M. Giblin-Davis & B. J. Center. 1993.

Resistance to the sting nematode. Crop Sci. 33:1066-1070.

Castle, S. J. 1992. Field and laboratory transmission of watermelon mosaic virus-2 and zucchini yellow mosaic by various aphid species. Phytopathology 82:235-240.

Chambliss, O. L. 1966. Inheritance and insect attractant properties of a bitter principle in watermelon Citrullus vulgaris Schrad. PhD Thesis, Purdue Univ., West Layfayette, IN.

Chung, J. H. 1967. Genetic control of varietal traits in watermelon, Citrullus vulgaris Schrad. MS Thesis, Kansas State Univ. Pittsburgh, KS.

Clark, M. F. & A. M. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunoabsorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.

Crall, J. M., G. W. Elmstrom & F. McCuistion. 1994. SSDL: A high quality icebox breeding line resistant to Fusarium wilt and Anthracnose. HortScience 29:707-708.

Davis R. F. & M. K. Mizuki. 1987. Detection of cucurbit virus in New Jersey. Plant Dis. 71:40-44.

Demski, J. W. & J. H. Chalkley. 1972. Effect of watermelon mosaic virus on watermelon. Plant Dis. Rptr. 58:195-198.

Demski, J. W. & G. Sowell, Jr. 1970. Susceptibility of Cucurbita pepo and Citrullus lanatus introduction to watermelon mosaic virus-2. Plant Dis. Rptr. 54:880-881.

De Sa, P. B. & E. W. Kitajima. 1991. Characterization of an isolate of watermelon mosaic virus-2 (WMV-2) from Brazil. Fitopatol. Bras. 16:217-223.

Dhaliwal, M. S. 1984. Diallel analysis of some agronomic characters of watermelon. SABRAO-J. 16:49-56.

Driscoll, C. J. 1986. Nuclear male sterility system in seed production of hybrid varieties. CRC Critical Rev. Plant Sci. 3:227-256.

Dyutin, K. E. 1990. A spontaneous watermelon mutant with male sterility. Cytology and Genetics (English translation of Russian journal title) 24(2):66-67.

Filor, A. I. 1939. Reaction of hermaphroditic varieties of cucurbits to self pollination. Proc. Linn. Acad. Agr. Sci. USSR.(Plant Breed. Abst. 10, 1940) 12:6-10.

Filov, A. T. & V. P. Toscev. 1968. Inheritance of sugar content in melons. Cytology and Genetics (English translation of Russian journal title) 2:144-151.

Fisher, H. U. & B. E. L. Lockhart. 1974. Serious losses in cucurbits caused by watermelon mosaic virus in Morocco. Plant Dis. Rptr. 58:143-146.

Gerber, R. S. 1969. Viruses infecting cucurbits in Queensland. Qd. J. Agr. Anim. Sci. 26:145-177.

Gillaspie, A. G. & J. M. Wright. 1993. Evaluation of Citrullus sp germplasm for resistance to watermelon mosaic virus 2. Plant Dis. 77:352-354.

Green, C. H. 1959. Effects of ploidy differences on horticultural characteristics and disease resistance in watermelons. PhD Thesis, Purdue Univ.

Green, C. H. & E. C. Stevenson. 1962. Effects of ploidy differences on horticultural characteristics in watermelon. Proc. Amer. Soc. Hort. Sci. 80:550-555.

Harpaz, J. 1982. Nonpesticidal control of vector borne virus, p. 1-44. In: K. F. Harris & K. Maramorosch (eds.). Pathogens, Vectors and Plant Disease: Approaches to Control. Academic Press, New York.

Hashizume, T., I. Shimamoto, Y. Harushima, M. Yur, T. Sato, T. Imai & M. Hirai. 1996. Construction of a linkage map for watermelon (Citrullus lanatus (Thunb.) Matsum & Nakai) using random amplified polymorphic DNA (RAPD). Euphytica 90:265-273.

Henderson, W. R. 1977. Effects of cultivar, polyploidy and reciprocal hybridization on character important in breeding triploid seedless watermelon hybrids. J. Amer. Soc. Hort. Sci. 102:293-297.

Henderson, W. R. 1989. Inheritance of orange flesh color in watermelon. Cucurbit Genet. Coop. 12:59-63.

Hensz, R. A. & H. C. Mohr. 1959. Functional male sterility in watermelon induced by chemical treatment. Proc. Amer.

Soc. Hort. Sci. 74:552-554.

Ho, I., Y. Wan & J. M. Widholm. 1990. The use of stomatal chloroplast number for rapid determination of ploidy level in maize. Plant Breed. 105:203-210.

Hochmuth, G., and G. Elmstrom. 1992. Cultural Practices pp.19-22. in: D. N. Maynard. (ed) Watermelon Production Guide for Florida. SP113. Florida Coop. Ext. Serv. Gainesville, FL.

Hopkins, D. & G. W. Elmstrom 1993. The effects of triploids on seed size and disease resistance in triploid watermelons. Proc. Fla. State Hortic. Soc. 100:281.

Hull, R. & J. W. Davies. 1992. Approaches to nonconventional control of plant virus diseases. Critical Rev. of Plant Sci. 11:17-33.

Inouye, T. 1964. A virus disease of pea caused by watermelon mosaic virus. Ber. Ohara. Inst. Biol. 12:133-143.

Ivanoff, S. S. & R. C. Albritton. 1963. Watermelon hybrids. Bull. Miss. Agric. Exp. Stn. 643:11.

Kaul, M. L. H. 1988. Male sterility in higher plants. Monogr. Theor. Appl. Gen. 10. Springer Verlag, New York.

Kihara, H. 1951. Triploid watermelons. Proc. Amer. Soc. Hort. Sci. 58:217-230.

Lecog, H., M. Pitrat & M. Clement. 1981. Identification and characterization d'un potyvirus provoquant la malade du rabougrissement janue de melon. Agronomie 1:827-834.

Lindberg, G. D., D. H. Hall & J. C. Walker. 1956. A study of melon and squash mosaic virus. Phytopathology 46:489-495.

Lopez-Abelia, D., R. H. E. Bradley & K. F. Harris. 1988. Correlation between stylet paths made during superficial probing and the ability of aphids to transmit non-persistent viruses. Adv. Dis. Vector Res. 5:251-285.

Love, S. L, B. B. Rhodes & P. E. Nugent. 1986. Controlled pollination transfer of a male-sterile gene from a diploid to a tetraploid watermelon line. Euphytica 35:633-638.

Lovisolo, O. 1980. Virus and viroid diseases of cucurbits. Acta. Hortic. 88:33-82.

Maelzer, D. A. 1986. Integrated control of insect vectors of plant-virus diseases, p. 483-512. In: G. D McClean, R. D. Garret & W. D. Ruesink (eds.). Plant Virus Epidemics. Academic Press, Australia.

Marco, K. S. 1993. Incidence of non-persistently transmitted viruses in pepper sprayed with whitewash, oil and insecticide, alone or combined. Plant Dis. 77:1119-1122.

Maxwell, K. 1992. Aiton: Seedless sales "Exploding." The Produce News, May 2, p.23.

Milne, K. S. & R. G. Grogan. 1969. Characterization of watermelon mosaic virus strains by serology and other properties. Phytopathology 59:809-818.

Mohr, H. C. 1942. A mutant leaf form in watermelon. Proc. Assoc. So. Ag. Workers 39:129-130.

Mohr, H. C. 1956. Mode of inheritance of the bushy growth characteristic in watermelon. Proc. Assoc. So. Ag. Workers 53:174.

Mohr, H. C. 1958. Bush desert king watermelon. Leaflet 397. Texas Agr. Expt. Sta.

Mohr, H. C. 1963. Utilization of the genetic character for short-internode in improvement of the watermelon. Proc. Amer. Soc. Hort. Sci. 82:454-459.

Mohr, H. C., H. T. Blackhurst & E. R. Jensen. 1955. F_1 hybrid watermelons from open-pollinated seed by use of a genetic marker. Proc. Amer. Soc. Hort. Sci. 65:399-404.

Mora-Aguilera, G. & S. E. Webb. 1993. Spatial patterns of watermelon mosaic virus epidemics. Proceedings of the 6th Int'l Cong. Of Pl Path. July 28-Aug. 6, Montreal Canada. 101 pp.

Morton, D. J. & R. E. Webb. 1963. Symptoms associated with watermelon mosaic virus in South Carolina. Plant Dis. Rptr. 47:772-773.

Munger, H. M., T. A. More & S. Awni. 1984. A preliminary report on screening watermelons for resistance to

watermelon mosaic virus 1 and 2. Cucurbit Genet. Coop. 7:61-62.

Murdock, B. A., N. H. Ferguson & B. B. Rhodes. 1990. Male-sterile (ms) from China apparently non-allelic to glabrous male-sterile gms watermelon. Cucurbit Genet. Coop. 13:45.

Nameth, S. T., F. L. Laemmlen & J. A. Dobbs. 1985. Viruses cause heavy melon losses in desert valleys. Cal. Ag. July-Aug., p 28-29.

Nameth, S. T., J. A. Dobbs, A. O. Paulus & F. L. Laemmlen. 1986. Cucurbit viruses of California an ever changing problem. Plant Dis. 70:8-13.

Navot N., M. Sarfatti & D. Zamir. 1990. Linkage relationships of genes affecting bitterness and flesh color in watermelon. J. Hered. 81(2):161-165.

Nelson, M. R. & D. M. Tuttle. 1969. The epidemiology of cucumber mosaic and watermelon mosaic 2 of cantaloupes in an arid climate. Phytopathology 59:849-856.

Novy, R. G. & J. P. Helgeson. 1994. Resistance to potato virus Y in somatic hybrids between Solanum etuberosum and S. tuberosum x S. berthaultii hybrid. Theor. Appl. Genet. 89:783-786.

Ortiz, R. & D. Vuylsteke. 1994. Inheritance of black sigatoka disease resistance in plantain-banana (Musa spp.) hybrids. Theor. Appl. Genet. 89:146-152.

Orton, W.A. 1911. The development of disease resistant varieties of plants. IV Conf. Int. Genetique, Paris, Comptes Rendus et Rapports p.247-265.

Pecker, I., R. Gabbay, F. X. Cunningham & J. Hirshberg. 1996. Cloning and characterization of the cDNA for lycopene B-cyclase from tomato reveals decrease in its expression during fruit ripening. Plant Mol. Biol. 31:807-819.

Perring, T. M., R. N. Royalty & C. A. Farrar. 1989. Floating row covers for the exclusion of virus vectors and the effect on disease incidence and yield of cantaloupe. J. Econ. Entomol. 82:1709-1715.

Poole, C. F. 1944. Genetics of cultivated cucurbits. J. Hered. 35:122-128.

Porter, D. R. 1937a. Inheritance of certain fruit and seed characters in watermelon. Hilgardia 10:489-509.

Porter, D. R. 1937b. Breeding high quality wilt resistant watermelons. Cal. Agr. Expt. Sta. Bul. 614:1-43.

Porter, D. R. & I. E. Melhus. 1932. The pathogenicity of Fusarium niveum E. F. S. and the development of wilt resistant strains of Citrullus vulgaris Schrad. Iowa Expt. Sta. Res. Bul. 149:123-184.

Powell, G. 1991. Cell membrane puncture during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. Ann. Appl. Biol. 119:313-321.

Provvidenti, R. 1986. Viral diseases of cucurbits and sources of resistance. Food and Fertilizer Tech. Center Taiwan Tech. Bul. No.93.

Provvidenti, R. & W. T. Schoeder. 1970. Epiphytotic of watermelon mosaic among Cucurbitaceae in central New York in 1969. Plant Dis. Rptr. 54:744-748.

Purcifull, D. E. & E. Hiebert. 1979. Serological distinctive of watermelon mosaic virus isolates 2 epidemics in Florida 1967-1977. Phytopathology 69:112-116.

Purcifull, D. E., G. W. Simone, C. A. Baker & E. Hiebert. 1988. Immunodiffusion tests for six viruses that infect cucurbits in Florida. Proc. Fla. State Hortic. Soc. 101:400-403.

Purcifull, D. E., E. Hiebert & J. Edwardson. 1984. Watermelon mosaic virus 2 No.293. In: Descriptions of Plant Viruses Commonw. Mycol. Inst./Assoc. Appl. Bio, Kew, England.

Raccah, B. 1986. Nonpersistent viruses: Epidemiology and control. Adv. Virus Res. 31:387-429.

Rao, M. K., K. U. Devi & A. Arundhati. 1990. Application of genic male sterility in plant breeding. Plant Breed. 105:1-25.

Ray D. T. & J. D. Sherman. 1988. Synaptic chromosome behavior of the gms mutant in watermelon. J. Hered. 79:397-399.

Rhem, S. 1952. Male-sterile plants by chemical treatment. Nature 170:38-39.

Rhodes, B. B. 1986. Genes affecting foliage color in watermelon. J. Hered. 77:134-135.

Rhodes, B. B., J. W. Adelberg & R. T. Nagata. 1988. Efforts to use gms gene to produce commercial seedless watermelons. International Symposium on Hort. Germplasm, Cultivated and Wild (Abstract). P. 171.

Schaffner, J. H. 1903. Atavism in watermelon. Ohio Nat. 3:370-371.

Shimotsuma, M. 1963. Cytological studies in the genus Citrullus. VII Inheritance of several characters in watermelons. Jpn. J. Breed. 13:235-240.

Shimotsuma, M. 1965. A survey of seedless watermelon breeding and its extension in Taiwan. Kihara Inst. for Biol. Res. Seiken Ziho 17:46-54.

Sidhu, A. S. 1977. Mode of inheritance of quality characters in watermelon. J. Res. Punjab Agr. Univ. 14(4):423-426.

Sidhu, A. S. 1982. Studies on a diallel cross of watermelon. Vegetable Science 9(1):34-41.

Smith, K. M. 1972. A Textbook of Plant Virus Diseases 3rd ed. Academic Press, New York. 684 pp.

Sowell G., Jr. & J. W. Demski. 1969. Susceptibility of watermelon cultivars to watermelon mosaic virus-2. Plant Dis. Rptr. 53:208-209.

Suzuki, Y. & C. V. Hall. 1971. Inheritance of melon weight, total soluble solids, seed size and number, and rind thickness and toughness in watermelon. HortScience 6/3 sect 2.

Toba, H. H. 1962. Studies on the host range of watermelon mosaic virus in Hawaii. Plant Dis. Rptr. 46:409-410.

Toba, H. H. 1963. Vector-virus relationships of watermelon mosaic virus and the green peach aphid Myzus persicae. J. Econ. Entomol. 56:200-205.

Toba, H. H., A. N. Kishaba, G. W. Bohn & H. Hield. 1977. Protecting muskmelons against aphid-borne viruses. Phytopathology 67:1418-1423.

Tomes, M. L., K. W. Johnson & M. Hess. 1963. The carotene pigment of certain red fleshed watermelons. Proc. Amer. Soc. Hort. Sci. 82:460-464.

Vallejo, R. L., W. W. Collins & J. B. Young. 1995. Inheritance of resistance to potato virus Y and potato virus X in hybrid Solanum phureja x S. stenotomum diploid potatoes. J. Hered. 86:89-93.

Van Regenmortel, M. H. 1971. Watermelon mosaic virus in description of plant viruses No. 63. Commonwealth Mycological Institute and Association of Applied Biologist. Kew, Surrey, England.

Van Regenmortel, M. H., J. Brandes & R. Bucks. 1962. Investigations on the properties of watermelon mosaic virus. Phytopathology 45:205-216.

Walker, M. N. 1933. Occurrence of watermelon mosaic virus. Phytopathology 23:74-744.

Warid, W. A. & A. A. Abd-El-Hafez. 1976. Inheritance of marker genes of leaf color and ovary shape in watermelon Citrullus vulgaris Schrad. Libyan J. Sci. 6:1-8.

Warid, W. A., K. R. Stino & A. A. Abd-El-Hafez. 1971. Seed germination, leaf color and plant sex as marker traits in polyploid breeding of watermelon. Libyan J. Agric. 1:13-18.

Watanabe, K. & T. Saito. 1987. Carotenoid pigments in red, orange and yellow fleshed fruits of watermelon (Citrullus vulgaris) cultivars. J. Japanese Soc. Hort. Sci. 56(1):45-50.

Watts, V. M. 1962. A marked male-sterile mutant in watermelon. Proc. Amer. Soc. Hort. Sci. 81:498-505.

Watts, V. M. 1967. Development of disease resistance and seed production in watermelon stocks carrying the ms gene. Proc. Amer. Soc. Hort. Sci. 91:579-583.

Webb, R. E. 1965. Luffa acutangula for separation and maintenance of watermelon mosaic virus 1 free of watermelon

mosaic virus. Phytopathology 55:1379-1380.

Webb, R. E. 1977. Resistance to watermelon mosaic virus-2 in Citrullus lanatus (Abstr.) Proc. Am. Phytopathol. Soc. 4:220.

Webb, R. E. 1992. Distribution of watermelon mosaic virus 2 in watermelon and acquisition of virus by aphids: Effects of plant age at inoculation (Abstr). Phytopathology 82:1173.

Webb, R. E., G. W., Bohn & H. A. Scott. 1965. Watermelon mosaic virus 1 and 2 in southern and western cucurbit production areas. Plant Dis Rptr. 49:532-535.

Webb, R. E. & S. B. Linda. 1993. Effects of oil and insecticide on epidemics of potyviruses in watermelon. Plant Dis. 77:869-874.

Webb, R. E. & H. A. Scott. 1965. Isolation and identification of watermelon mosaic viruses 1 and 2. Phytopathology 55:895-900.

Welch, A. & I. E. Melhus. 1942. Wilt resistance in F_1 hybrid watermelons. Phytopathology 32:181-182.

Winstead, N. N., M. J. Goode, and W. S. Barham. 1959. Resistance in Watermelon to Colletotrichum lagenarium race 1, 2, and 3. Plant Dis. Rptr. 43:570-577

Wittwer, S. H. 1953. Chemical control of flower sex ratio in cucurbits. Abstracts of Papers, Semicentennial Meeting Amer. Soc. Hort. Sci.

Xia, X., Y. Liu, W. Liu & A. Chen. 1988. Selection of watermelon line G17AB. J. Shengyang Agr. Univ. 19:9-13.

Xian, Zhang. 1991. Development of male-sterile watermelon line and utilization. 23rd Int'l Hort. Congress Abstract 1193, p. 91.

Yamamoto, T. 1986. Infection cycle of watermelon mosaic virus. J. A. R. Q. 19:259-265.

Yamamoto, T., N. Ishii, T. Katsube & M. Sorin. 1982. Transmission of watermelon mosaic virus by aphid species. Jpn. J. Appl. Entomol. Zool. 26:218-223.

Zhang, X. P., B. B. Rhodes, W. V. Baird, H. T. Skorupska & W.C. Bridges. 1996. Development of genic male-sterile watermelon lines with delayed-green seedling marker. HortScience 31:123-126.

Zhang, X. P. & M. Wang. 1990. A genetic male-sterile (ms) watermelon from China. Cucurbit Genet. Coop. 13:45.

APPENDIX

Table A-1. Distribution of number of fruits classified for flesh color in fruits from Calsweet 4x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	0	2	1
Medium red with orange	0	0	0
Medium red	0	1	7
Light red with orange	0	1	0
Light red	1	9	11
Pink	43	47	39

Table A-2. Distribution of number of fruits classified for flesh color in fruits from Elisa 4x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	8	11	9
Medium red with orange	10	0	9
Medium red	36	42	32
Salmon	7	14	15
Light red with orange	4	2	9
Light red	10	14	4
Pink	2	0	0

Table A-3. Distribution of number of fruits classified for flesh color in fruits from Calsweet 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	2	8	18
Medium red with orange	1	3	23
Medium red	20	29	4
Light red with orange	6	0	3
Light red	17	22	6

Table A-4. Distribution of number of fruits classified for flesh color in fruits from Elisa 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	5	9	7
Medium red with orange	21	1	15
Medium red	11	19	14
Salmon	14	31	12
Light red with orange	3	5	6
Pink	20	22	26

Table A-5. Distribution of number of fruits classified for flesh color in fruits from Elisa 2x X Calsweet 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	6	3	1
Medium red with orange	2	1	9
Medium red	18	35	20
Salmon	2	1	2
Light red with orange	4	18	7
Light red	30	12	25
Pink	12	10	2

Table A-6. Distribution of number of fruits classified for flesh color in fruits from Calsweet 2x X Elisa 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	2	5	11
Medium red with orange	3	8	6
Medium red	30	41	32
Salmon	6	0	2
Light red with orange	4	1	8
Light red	25	23	14
Pink	7	12	6

Table A-7. Distribution of number of fruits classified for flesh color in fruits from Elisa 4x X Elisa 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	8	5	8
Medium red with orange	10	3	11
Medium red	42	39	30
Salmon	0	11	10
Light red with orange	1	4	4
Light red	24	26	20
Pink	3	5	0

Table A-8. Distribution of number of fruits classified for flesh color in fruits from Elisa 4x X Calsweet 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	4	18	19
Medium red with orange	7	6	3
Medium red	53	35	47
Salmon	0	1	1
Light red with orange	0	3	5
Light red	17	15	5
Pink	1	2	0

Table A-9. Distribution of number of fruits classified for flesh color in fruits from Calsweet 4x X Elisa 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	1	0	1
Medium red with orange	0	0	0
Medium red	18	20	13
Deep pink	0	1	2
Salmon	0	0	0
Light red with orange	2	0	1
Light red	19	15	19
Pink	20	9	11

Table A-10. Distribution of number of fruits classified for flesh color in fruits from Calsweet 4x X Calsweet 2x plants inoculated with WMV-2.

Flesh color	Treatment		
	Inoculated	Non-inoculated	Open
Deep red	0	4	5
Medium red with orange	0	1	2
Medium red	7	23	24
Salmon	2	0	0
Light red with orange	0	1	0
Light red	14	6	26
Pink	22	31	11

BIOGRAPHICAL SKETCH

Fred Talmadge McCuistion was born on June 3, 1963, in Little Rock, Arkansas, and was raised in Rutherford, New Jersey. In 1985 he received his Bachelor of Arts degree in chemistry with a minor in biology and a secondary teaching certification from The Kings College in Briarcliff Manor, New York. Subsequently, he taught chemistry at Yorktown H.S. in Yorktown, N.Y. for one year, then returned to school at Virginia Polytechnic Institute where he obtained a Master of Science degree in agronomy in 1990. While writing the M.S., he worked for DNA Plant Technology in Cinnaminson N.J. for two years. He decided to work on a doctoral degree at the University of Florida when a scholarship was offered there. Upon completion of the degree, he anticipates a postdoctorate position at North Carolina State University.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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